MarrowMAX™ Bone Marrow Medium

P R O D U C T N E W S

- ***Optimized for cytogenetic analysis***
- ***High mitotic index***
- ***Excellent chromosomal morphology***
- ***Consistent performance***

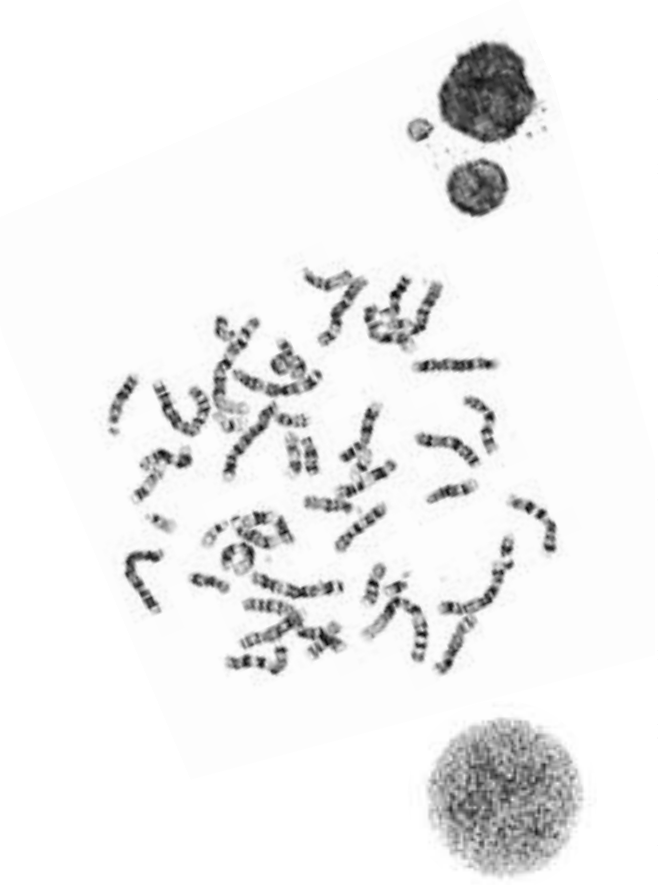


Figure 1. Chromosome spread from normal bone marrow cells. Cells were cultured in MarrowMAX™ Medium for 24 h.

Analysis of human tumors and hematopoietic cells for diagnosis of malignancies is a rapidly growing area of clinical cytogenetics. For the short-term culture of bone marrow, peripheral blood, and hematopoietic cells required for these analyses, many labs use Giant Cell Tumor (GCT) conditioned media containing a variety of hematopoietic growth factors to supplement serum-containing cultures. However, it is difficult to achieve consistent high levels of analyzable cells with commercially available or homemade formulations supplemented with GCT.

GIBCO™ MarrowMAX™ Bone Marrow Medium is a fully supplemented medium developed specifically to support bone marrow and peripheral blood cell culture for *in vitro* cytogenetic analysis of hematological disease.

MarrowMAX™ Bone Marrow Medium contains a novel human stromal cell conditioned medium. This conditioned medium is composed of a unique blend of hematopoietic growth factors for optimal cell growth. The medium is manufactured under strict controls ensuring consistent performance and superior chromosomal morphology *(figure 1)*. Using MarrowMAX™ Medium results in cultures with a high mitotic index and an increased number of analyzable cells.

Superior Performance

- Outperforms commercially available media containing GCT *(figure 2, reverse)*.
- Higher mitotic index and superior chromosomal morphology.
- Consistent lot-to-lot performance *(figure 3, reverse)*.

Convenience

- Complete, ready-to-use medium.
- Fully supplemented with serum, antibiotics, and L-glutamine.
- Store either frozen or refrigerated.

Quality Assurance

- Manufactured in compliance with the FDA's Quality System Regulation (cGMP) and the current requirements of ISO 9001.
- Application tested by an independent certified cytogenetics laboratory using human bone marrow cells.
- Extended shelf life of 18 months when stored unopened at –20˚C and 60 days stored at 4˚C.

GIBCO™
cell culture

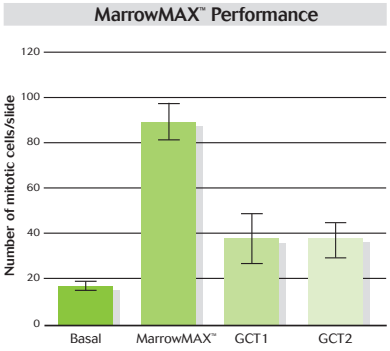


Figure 2. Comparison of media for stimulation of mitotic cells. Cells were cultured in basal medium without conditioned medium (Basal), MarrowMAX™ Medium which contains stromal cell-conditioned medium (MarrowMAX™), Supplier 1 Medium containing GCT-conditioned medium (GCT 1), and Supplier 2 Medium containing GCT-conditioned medium (GCT 2). Mitotic cells were assayed 24 h after plating. Results are mean ± SEM for N = 10 with up to 30 donors.

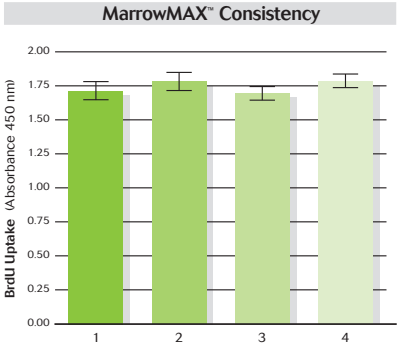


Figure 3. MarrowMAX™ Medium consistency. Normal bone marrow mononuclear cells were seeded at 1 × 10^5 cells/ml in 4 different lots of MarrowMAX™ Medium. Results are mean ± SEM for N = 10.

Ordering Information

Description	Cat. No.	Size
MarrowMAX™ Bone Marrow Medium** (contains gentamicin)	12260-014	100 ml
Related Products		
Complete Media		
AmnioMAX™-II Complete Medium (contains gentamicin)	11269-016	100 ml
AmnioMAX™-C100 Complete Medium (system) — The system contains both the medium (90 ml) and the supplement (15 ml) (supplement contains gentamicin)	12558-011	1 Set
AmnioMAX™-C100 Basal Medium, liquid	17001-082 17001-074	90 ml 450 ml
AmnioMAX™-C100 Supplement, liquid (supplement contains gentamicin)	12556-015 12556-023	15 ml 75 ml
PB-MAX™ Karyotyping Medium (supplement contains gentamicin)	12557-013 12557-021	100 ml 500 ml
Reagents		
KaryoMAX® Colcemid® Solution, liquid (10 µg/ml), in HBSS	15210-040	10 ml
KaryoMAX® Colcemid® Solution, liquid (10 µg/ml), in PBS	15212-012	10 ml
KaryoMAX® Giemsa Stain Stock Solution	10092-013	100 ml
Gurr Buffer Tablets (pH 6.8)*	10582-013	50 × 1 L
Phytohemagglutinin (M Form) (PHA), lyophilized*	10576-015	10 ml

See Chapter 3 of the 2003 GIBCO™ Catalog for more related products.

Invitrogen™ life technologies

www.invitrogen.com

GIBCO™ cell culture

Corporate Headquarters: Invitrogen Corporation • 1600 Faraday Avenue • Carlsbad, California 92008 U.S.A.
Tel: 1 760 603 7200 • Tel (Toll Free): 1 800 955 6288 • Toll Free Fax: 1 800 331 2286 • E-mail: tech_service@invitrogen.com
European Headquarters: Invitrogen Ltd • 3 Fountain Drive • Inchinnan Business Park • Paisley PA4 9RF, UK
Tel: +44(0) 141 814 6100 • Fax: +44(0) 141 814 6260 • E-mail: eurotech@invitrogen.com

applied biosystems
by life technologies

GeneScan™ 500 ROX™ Size Standard

Catalog Number 401734

Pub. no. 4340060 **Rev.** F

Contents†	Size	Storage conditions
16 ROX™ dye-labeled, single-stranded DNA fragments	2 X 200 µL (800 reactions‡)	2–8°C for 1 year from date of manufacture. Do not freeze.

† With the retirement of the ABI PRISM® 377 DNA Sequencer this kit no longer contains the gel loading buffer. If you need to continue to purchase the loading buffer, it is available as a separate part (Cat. no. 402055).

‡ 800 reactions when using the recommended loading amount of 0.5 µL.

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product description

GeneScan™ 500 ROX™ Size Standard is an internal lane size standard developed for use with fluorescence-based DNA electrophoresis systems from Life Technologies. The use of an internal lane size standard enables automated data analysis and is also essential for achieving high run-to-run precision in sizing DNA fragments by electrophoresis. GeneScan™ 500 ROX™ Size Standard is designed for sizing DNA fragments in the 35–500 bp range and provides 16 single-stranded, ROX™ dye-labeled fragments of 35, 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490, and 500 bases. Each of the DNA fragments is labeled with a proprietary fluorophore, which results in a single peak when run under both denaturing and non-denaturing conditions.

Instructions for use

Sample preparation

1. Before use, mix the contents of each tube thoroughly and centrifuge briefly to collect the liquid at the bottom of the tube. Typical loading cocktails are as follows:

	Electrophoresis system			
Component	**310**	**3130/3100 Series**	**3500 Series**	**3730 Series**
Sample	0.5 µL	0.5 µL	0.5 µL	0.5 µL
GeneScan™ 500 ROX™ Size Standard	0.5 µL	0.25 µL	0.25 µL	0.5 µL
Hi-Di™ Formamide (Cat. no. 4311320)†	9.0 µL	9.25 µL	9.25 µL	9.0 µL

† Not included in this kit.

Note: We highly recommend using the ratios of DNA sample (PCR product) and size standard presented in the table as a starting point only. Optimize the ratios if necessary, based on your experimental results. Also, see guidelines in the following section.

IMPORTANT! For HID applications, please follow protocol volumes provided with those application kits.

2. Heat the loading cocktail for 3 minutes at 95°C.
3. Immediately chill on ice for a few minutes and load samples.

Guidelines

Optimize your analysis based on the following information about use of this standard on capillary electrophoresis instruments:

- The 250-bp peak is sensitive to small temperature variations. The 250-bp fragment should not be used when defining the size standard in GeneMapper® Software.
- The 340-bp peak is subject to large temperature variations.
- Fragment analysis primer peaks can often interfere with the detection of the 35-bp peak.

Note: Discard any unused reagent that has been diluted in Hi-Di™ Formamide.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

For support visit **lifetechnologies.com/support** or email **techsupport@lifetech.com**

lifetechnologies.com

December 2013

7500 Real-Time PCR Systems Spectral Calibration Kit II

Catalog Number 4351151

Pub. No. 4351155 **Rev.** B

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Contents and storage

Contents	Amount	Storage
Spectral Calibration Plates sealed with optical covers	3	–25°C to –15°C

Related documentation

For detailed information on instrument setup and the calibration process, refer to the *Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide* (Pub. no. 4347828).

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

For support visit **thermofisher.com/support** or email **techsupport@lifetech.com**

1 December 2015

ThermoFisher SCIENTIFIC

SAFETY DATA SHEET

(In accordance with COMMISSION REGULATION (EU) No 830/2015)

SECTION 1: Identification of the substance/mixture and of the company/undertaking

Product identifier

Product code	10092013
Product name	KaryoMAX™ Giemsa Stain Solution
Chemical Name	Not Applicable
REACH registration number	No registration number is given yet for this substance / substances in this mixture since the annual import quantity is less than one tonnage per annum or the transition period for its registration according to Article 23 of REACH has not yet expired.

Relevant identified uses of the substance or mixture and uses advised against

Relevant identified uses	For in vitro diagnostic use
Use Description Code	SU20 - Health services, PC21 - Laboratory chemicals, SU22 - Public domain (administration, education, entertainment, services, craftsmen), PROC15 - Use as a laboratory reagent
Uses advised against	Not for consumer use.

Details of the supplier of the safety data sheet

Manufacturer/Supplier
LIFE TECHNOLOGIES EUROPE BV
KWARTSWEG 2
2665 NN BLEISWIJK
NETHERLANDS
31-(0)180 392 400
Email: MSDS@lifetech.com

24 hour Emergency Response for Hazardous Materials [or Dangerous Goods] Incident. Spill, Leak, Fire, Exposure, or Accident. Call CHEMTREC	Within the USA + Canada: 1-800-424-9300 and +1 703-527-3887 Outside the USA + Canada: +1 703-741-5970

Country specific Emergency Number (if available):

CHEMTREC Ireland (Dublin)	+(353)-19014670 (Greeting Language: English and Irish)
CHEMTREC UK (London)	+(44)-870-8200418 (Greeting Language: English)

SECTION 2: Hazards identification

Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008 [CLP]

Physical hazards

Flammable liquids	Category 2

Health hazards

Acute oral toxicity	Category 3
Acute dermal toxicity	Category 3
Acute inhalation toxicity	Category 3
Specific target organ toxicity - Single exposure	Category 1

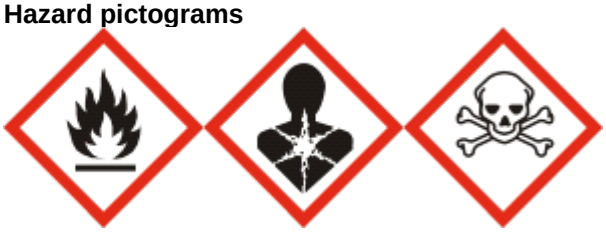
Environmental Hazards
Not classified

Additional information
Not Applicable

Label elements

Labelling according to Regulation (EC) No 1272/2008 [CLP]

Hazard pictograms



Signal word
Danger

Hazard Statements
H225 - Highly flammable liquid and vapour
H301 - Toxic if swallowed
H311 - Toxic in contact with skin
H331 - Toxic if inhaled
H370 - Causes damage to organs

EU Specific Hazard Statements
Not Applicable

Precautionary Statements

Prevention
P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking
P241 - Use explosion-proof electrical/ ventilating/ lighting/ equipment
P261 - Avoid breathing dust/fume/gas/mist/vapours/spray
P264 - Wash hands thoroughly after handling
P280 - Wear protective gloves/protective clothing/eye protection/face protection

Response

P301 + P310 - IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician
P302 + P352 - IF ON SKIN: Wash with plenty of soap and water
P304 + P340 - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
P308 + P311 - IF exposed or concerned: Call a POISON CENTER or doctor
P370 + P378 - In case of fire: Use dry sand, dry chemical or alcohol-resistant foam for extinction

Storage
P403 + P235 - Store in a well-ventilated place. Keep cool
P403 + P233 - Store in a well-ventilated place. Keep container tightly closed

Disposal
P501 - Dispose of contents/ container to an approved waste disposal plant

Other hazards
Not Applicable

SECTION 3: Composition/information on ingredients

Component	CAS-No.	EINECS-No.	Weight percent	REACH registration number	Classification according to Regulation (EC) No 1272/2008 [CLP]
Methyl alcohol 67-56-1 (60-100)	67-56-1	200-659-6	60-100	01-2119392409-28-XXXX	Flam. Liq. 2 - H225Tox. 3 - H301Tox. 3 - H311Tox. 3 - H331SE 1 - H370

SECTION 4: First aid measures

Description of first aid measures

Skin contact Rinse with plenty of water. Immediate medical attention is not required.
Eye contact Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.
INGESTION Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.
Inhalation Not expected to be an inhalation hazard under anticipated conditions of normal use of this material. Consult a physician if necessary.
Notes to Physician Treat symptomatically.

Most important symptoms and effects, both acute and delayed
H225 - Highly flammable liquid and vapour H301 + H311 + H331 - Toxic if swallowed, in contact with skin or if inhaled
H370 - Causes damage to organs

Indication of any immediate medical attention and special treatment needed
IF SWALLOWED: Call a POISON CENTRE or doctor/physician if you feel unwell. IF exposed or concerned: Get medical advice/attention. IF INHALED: Remove person to fresh air and keep comfortable for breathing.

SECTION 5: Firefighting measures

Extinguishing media

Suitable Extinguishing Media	Water spray. Carbon dioxide (CO2). Foam. Dry chemical.
Unsuitable Extinguishing Media	No information available.

Special hazards arising from the substance or mixture Not known.

Advice for fire-fighters Standard procedure for chemical fires.

SECTION 6: Accidental release measures

Personal precautions, protective equipment and emergency procedures
Ensure adequate ventilation. Always wear recommended Personal Protective Equipment. Use personal protection equipment. See Section 8 for more detail.

Environmental precautions
No special environmental precautions required. Avoid discharge into drains and waterways whenever possible.

Methods and material for containment and cleaning up
Soak up with inert absorbent material.

Reference to other sections
See section 8 for more information.

SECTION 7: Handling and storage

Precautions for safe handling
Use personal protective equipment as required. No special handling advices are necessary.

Conditions for safe storage, including any incompatibilities
Keep in a dry, cool and well-ventilated place. Keep in properly labelled containers.

Specific end use(s)
For in vitro diagnostic use.

SECTION 8: Exposure controls/personal protection

Control parameters

Chemical Name	EU OEL (TWA)	EU OEL (STEL)	EU Skin Notation
Methyl alcohol 67-56-1	200 ppm 260 mg/m³ Skin	None	None

Chemical Name	Austria	Belgium (TWA)	Denmark (TWA)	Finland OEL (TWA)
Methyl alcohol 67-56-1	200 ppm 260 mg/m³	200 ppm 266 mg/m³	200 ppm 260 mg/m³	200 ppm 270 mg/m³

Chemical Name	France OEL (VME)	Germany OEL (TWA)	Ireland (TWA)	Italy OEL (TWA)
Methyl alcohol 67-56-1	200 ppm 260 mg/m³	200 ppm exposure factor 4 270 mg/m³ exposure factor 4	200 ppm 260 mg/m³	200 ppm 260 mg/m³

Chemical Name	Sweden - Occupational Exposure Limits - TLVs (LLVs)	Netherlands OEL (MAC)	Spain OEL (TWA)	United Kingdom
Methyl alcohol 67-56-1	200 ppm LLV; 250 mg/m³ LLV	133 mg/m³ 100 ppm	200 ppm 266 mg/m³	200 ppm TWA; 266 mg/m³ TWA

Chemical Name	European Union	France OEL (VME)	Germany OEL (TWA)
Methyl alcohol 67-56-1	None	200 ppm 260 mg/m³	200 ppm exposure factor 4 270 mg/m³ exposure factor 4

Chemical Name	Italy OEL (TWA)	Portugal	Netherlands OEL (MAC)	Finland OEL (TWA)
Methyl alcohol 67-56-1	200 ppm 260 mg/m³	None	133 mg/m³ 100 ppm	200 ppm 270 mg/m³

Chemical Name	Austria	Denmark	Poland	Switzerland
Methyl alcohol 67-56-1	200 ppm 260 mg/m³	None	None	None

Chemical Name	Ireland	Norway	Lithuania OEL (TWA)	Spain OEL (TWA)
Methyl alcohol 67-56-1	None	None	200 ppm 260 mg/m³	200 ppm 266 mg/m³

Engineering measures Ensure adequate ventilation, especially in confined areas.

Exposure controls

Personal protection equipment

Respiratory protection In case of insufficient ventilation wear respirators and components tested and approved under appropriate government standards.

Hand Protection Wear suitable gloves. Glove material: Compatible chemical-resistant gloves.

Eye protection Tight sealing safety goggles.

Skin and body protection Wear suitable protective clothing.

Hygiene measures Handle in accordance with good industrial hygiene and safety practice.

Environmental exposure controls
No special environmental precautions required.

SECTION 9: Physical and chemical properties

Information on basic physical and chemical properties

Appearance	Liquid	
Odour	no data available	
pH	No data available	
Melting point / melting range	**°C** Mixture has not been tested	**°F** Mixture has not been tested
Boiling point / boiling range	**°C** Mixture has not been tested	**°F** Mixture has not been tested
Flash point	**°C** <23	**°F** <73.4
Autoignition temperature	**°C** Mixture has not been tested	**°F** Mixture has not been tested
Decomposition temperature	**°C** Mixture has not been tested	**°F** Mixture has not been tested
Evaporation rate	No data available	
Flammability (solid, gas)	No data available	
Upper explosion limit	Mixture has not been tested	
Lower explosion limit	Mixture has not been tested	
Vapour Pressure	Mixture has not been tested	
Relative density	Mixture has not been tested	
Specific gravity	No data available	
Solubility	no data available	
Partition coefficient: n-octanol/water	No data available	
Explosive properties	Mixture has not been tested	

OTHER INFORMATION No data available

SECTION 10: Stability and reactivity

Reactivity None known.

Chemical stability Stable under normal conditions.

Possibility of hazardous reactions Hazardous reaction has not been reported.

Conditions to Avoid No information available.

Incompatible Materials No dangerous reaction known under conditions of normal use.

Hazardous decomposition products No data available.

SECTION 11: Toxicological information

Information on toxicological effects

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
Methyl alcohol	= 6200 mg/kg (Rat)	No data available	=64000ppm(Rat) =22500ppm(Rat)

Principal Routes of Exposure, Potential health effects

Irritation Conclusive but not sufficient for classification

Corrosivity Conclusive but not sufficient for classification

Sensitisation Conclusive but not sufficient for classification

STOT - Single Exposure Target organ(s): Respiratory system, Central Nervous System (CNS).

STOT - Repeated Exposure Conclusive but not sufficient for classification

Carcinogenicity Conclusive but not sufficient for classification

Mutagenicity Conclusive but not sufficient for classification

Reproductive toxicity Conclusive but not sufficient for classification

Aspiration Hazard Conclusive but not sufficient for classification

SECTION 12: Ecological information

Toxicity
The environmental impact of this product has not been fully investigated.

Chemical Name	Freshwater Algae Data	Water Flea Data	Freshwater Fish Species Data	Microtox Data	log Pow
Methyl alcohol	No data available	No data available	No data available	No data available	logPow-0.77

Persistence and degradability No information available.

Bioaccumulative potential No information available.

Results of PBT and vPvB assessment
No information available.

Other adverse effects No information available.

SECTION 13: Disposal considerations

Waste treatment methods
The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique. Disposal of this product, its solutions or of any by-products, shall comply with the requirements of all applicable local, regional or national/federal regulations.

SECTION 14: Transport information

IATA / ADR / DOT-US / IMDG
Classified as dangerous in the meaning of transport regulations.

UN Number	1230
UN proper shipping name	Methanol
Transport hazard class(es)	3(6.1)
Packing group	II
Environmental Hazards	Not Hazardous
Special precautions for user	Not Applicable

Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code
Not Applicable.

SECTION 15: Regulatory information

Safety, health and environmental regulations/legislation specific for the substance or mixture

Substances of Very High Concern
None.

Restricted substances under EC 1907/2006, Annex XVII
None.

Substances listed under Annex I of Regulation (EC) No 689/2008
None.

Restricted substances under Annex V of Regulation (EC) No 689/2008
None.

Substances under Regulation (EC) No 850/2004 of the European Parliament and of the Council of 29 April 2004 on persistent organic pollutants and amending Directive 79/117/EEC
None.

German Water hazard classes (Wassergefährdungsklassen)

Chemical Name	Weight percent	Germany - Water Classification (VwVwS) - Annex 1	Germany - Water Classification (VwVwS) - Annex 2 - Water Hazard Classes	Germany - Water Classification (VwVwS) - Annex 3
Methyl alcohol	60-100		hazard class 1 - low hazard to waters	hazard class 2 - hazard to waters

Other International Inventories

Chemical Name	EINECS (European Union)	ELINCS (European List of Notified Chemical Substances)	ENCS (Japan)	PICCS (Philippines)
Methyl alcohol	Listed	-	Listed	Listed

Chemical Name	AICS (Australia)	South Korea (KECL)	Canada (DSL)	NDSL
Methyl alcohol	Listed	Listed	Listed	-

Chemical Safety Assessment
No Chemical safety assessment has been carried out.

SECTION 16: Other information

Reason for revision Update according to Commission Regulation (EU) No 453/2010.
Revision number 2
Revision date 04-Sep-2017

References
• ECHA: http://echa.europa.eu/
• TOXNET: http://toxnet.nlm.nih.gov/
• eChemPortal: http://www.echemportal.org/
• LOLI database: https://www.chemadvisor.com/loli-database

Classification and procedure used to derive the classification for mixtures according to Regulation (EC) 1272/2008 [CLP]:

Flammable liquids	Category 2	Calculation method
Acute oral toxicity	Category 3	Calculation method
Acute dermal toxicity	Category 3	Calculation method
Acute inhalation toxicity	Category 3	Calculation method
Specific target organ toxicity - Single exposure	Category 1	Calculation method

"The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS SDS DOES NOT CONSTITUTE A WARRANTY, EXPRESSED OR IMPLIED,INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE"

SAFETY DATA SHEET

(In accordance with COMMISSION REGULATION (EU) No 453/2010)

SECTION 1: Identification of the substance/mixture and of the company/undertaking

Product identifier

Product code	10575090
Product name	KaryoMAX® Potassium Chloride Solution
Chemical Name	Not applicable
REACH registration number	No registration number is given yet for this substance / substances in this mixture since the annual import quantity is less than one tonnage per annum or the transition period for its registration according to Article 23 of REACH has not yet expired.

Relevant identified uses of the substance or mixture and uses advised against

Relevant identified uses	For in vitro diagnostic use
Use Description Code	SU20 - Health services
Uses advised against	Not for consumer use.

Details of the supplier of the safety data sheet

Manufacturer/Supplier
LIFE TECHNOLOGIES EUROPE BV
KWARTSWEG 2
2665 NN BLEISWIJK
NETHERLANDS
31-(0)180 392 400
Email: MSDS@lifetech.com

24 hour Emergency Response for Hazardous Materials [or Dangerous Goods] Incident. Spill, Leak, Fire, Exposure, or Accident. Call CHEMTREC	Within the USA + Canada: 1-800-424-9300 and +1 703-527-3887 Outside the USA + Canada: +1 703-741-5970

Country specific Emergency Number (if available):

CHEMTREC Ireland (Dublin)	+(353)-19014670 (Greeting Language: English and Irish)
CHEMTREC UK (London)	+(44)-870-8200418 (Greeting Language: English)

SECTION 2: Hazards identification

Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008 [CLP]

Physical hazards
Not Hazardous

Health hazards
Not Hazardous

Environmental Hazards
Not Hazardous

Additional information
Not applicable

Label elements

Labelling according to Regulation (EC) No 1272/2008 [CLP]

Hazard pictograms
None

Signal word
None

hazard statements
Not applicable

EU Specific Hazard Statements
Not applicable

Precautionary Statements

Prevention
Not applicable

Response
Not applicable

Storage
Not applicable

Disposal
Not applicable

Other Hazards
This mixture does not contain any substances that are assessed to be a PBT or a vPvB

SECTION 3: Composition/information on ingredients

The product contains no substances which at their given concentration, are considered to be hazardous to health.

SECTION 4: First aid measures

Description of first aid measures

Skin contact	Rinse with plenty of water. Immediate medical attention is not required.
Eye contact	Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.
INGESTION	Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.
Inhalation	Not expected to be an inhalation hazard under anticipated conditions of normal use of this material. Consult a physician if necessary.
Notes to Physician	Treat symptomatically.

Most important symptoms and effects, both acute and delayed
Not applicable

Indication of any immediate medical attention and special treatment needed
None.

SECTION 5: Firefighting measures

Extinguishing media

Suitable Extinguishing Media	Water spray. Carbon dioxide (CO2). Foam. Dry chemical.
Unsuitable Extinguishing Media	No information available.

Special hazards arising from the substance or mixture Not known.

Advice for fire-fighters Standard procedure for chemical fires.

SECTION 6: Accidental release measures

Personal precautions, protective equipment and emergency procedures
Ensure adequate ventilation. Always wear recommended Personal Protective Equipment. Use personal protection equipment. See Section 8 for more detail.

Environmental precautions
No special environmental precautions required.

Methods and material for containment and cleaning up
Soak up with inert absorbent material.

Reference to other sections
See section 8 for more information.

SECTION 7: Handling and storage

Precautions for safe handling
Use personal protective equipment as required. No special handling advices are necessary.

Conditions for safe storage, including any incompatibilities
Keep in a dry, cool and well-ventilated place. Keep in properly labelled containers.

Specific end use(s)
For in vitro diagnostic use.

SECTION 8: Exposure controls/personal protection

Control parameters

Exposure Limits Contains no substances with occupational exposure limit values.

Engineering measures Ensure adequate ventilation, especially in confined areas.

Exposure controls

Personal protection equipment

Respiratory protection In case of insufficient ventilation wear respirators and components tested and approved under appropriate government standards.

Hand Protection Wear suitable gloves. Glove material: Compatible chemical-resistant gloves.

Eye protection Tight sealing safety goggles.

Skin and body protection Wear suitable protective clothing.

Hygiene measures Handle in accordance with good industrial hygiene and safety practice.

Environmental exposure controls
No special environmental precautions required.

SECTION 9: Physical and chemical properties

Information on basic physical and chemical properties

Appearance	Liquid	
Odour	no data available	
pH	6-8	
Melting point / melting range	**°C** Mixture has not been tested	**°F** Mixture has not been tested
Boiling point / boiling range	**°C** Mixture has not been tested	**°F** Mixture has not been tested
Flash point	**°C** Mixture has not been tested	**°F** Mixture has not been tested
Autoignition temperature	**°C** Mixture has not been tested	**°F** Mixture has not been tested
Decomposition temperature	**°C** Mixture has not been tested	**°F** Mixture has not been tested
Evaporation rate	No data available	
Flammability (solid, gas)	No data available	
Upper explosion limit	Mixture has not been tested	
Lower explosion limit	Mixture has not been tested	
Vapour Pressure	Mixture has not been tested	
Relative density	Mixture has not been tested	
Specific gravity	No data available	
Solubility	no data available	
Partition coefficient: n-octanol/water	No data available	
Explosive properties	Mixture has not been tested	

OTHER INFORMATION No data available

SECTION 10: Stability and reactivity

Reactivity None known.

Chemical stability Stable under normal conditions.

Possibility of hazardous reactions Hazardous reaction has not been reported.

Conditions to Avoid No information available.

Incompatible Materials No dangerous reaction known under conditions of normal use.

Hazardous decomposition products No data available.

SECTION 11: Toxicological information

Information on toxicological effects

There is no evidence available indicating acute toxicity.

Principal Routes of Exposure, Potential health effects

Irritation	Conclusive but not sufficient for classification
Corrosivity	Conclusive but not sufficient for classification
Sensitisation	Conclusive but not sufficient for classification
STOT - Single Exposure	Conclusive but not sufficient for classification
STOT - Repeated Exposure	Conclusive but not sufficient for classification
Carcinogenicity	Conclusive but not sufficient for classification
Mutagenicity	Conclusive but not sufficient for classification
Reproductive toxicity	Conclusive but not sufficient for classification
Aspiration Hazard	Conclusive but not sufficient for classification

SECTION 12: Ecological information

Toxicity
Contains no substances known to be hazardous to the environment or not degradable in waste water treatment plants.

Persistence and degradability No information available.

Bioaccumulative potential No information available.

Results of PBT and vPvB assessment
This mixture does not contain any substances that are assessed to be a PBT or a vPvB.

Other adverse effects No information available.

SECTION 13: Disposal considerations

Waste treatment methods
The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique. Disposal of this product, its solutions or of any by-products, shall comply with the requirements of all applicable local, regional or national/federal regulations.

SECTION 14: Transport information

IATA / ADR / DOT-US / IMDG
Not regulated in the meaning of transport regulations.

UN Number	Not applicable
UN proper shipping name	Not applicable
Transport hazard class(es)	Not applicable
Packing group	Not applicable
Environmental Hazards	Not applicable
Special precautions for user	Not applicable

Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code
Not applicable.

SECTION 15: Regulatory information

Safety, health and environmental regulations/legislation specific for the substance or mixture

Substances of Very High Concern
None.

Restricted substances under EC 1907/2006, Annex XVII
None.

Substances listed under Annex I of Regulation (EC) No 689/2008
None.

Restricted substances under Annex V of Regulation (EC) No 689/2008
None.

Substances under Regulation (EC) No 850/2004 of the European Parliament and of the Council of 29 April 2004 on persistent organic pollutants and amending Directive 79/117/EEC
None.

German Water hazard classes (Wassergefährdungsklassen)
Not classified.

Other International Inventories
No information available

Chemical Safety Assessment
No Chemical safety assessment has been carried out.

SECTION 16: Other information

Reason for revision Update according to Commission Regulation (EU) No 453/2010.
Revision number 2
Revision date 19-Dec-2016

References
• ECHA: http://echa.europa.eu/
• TOXNET: http://toxnet.nlm.nih.gov/
• eChemPortal: http://www.echemportal.org/
• LOLI database: https://www.chemadvisor.com/loli-database

Classification and procedure used to derive the classification for mixtures according to Regulation (EC) 1272/2008 [CLP]:
Not classified

"The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS SDS DOES NOT CONSTITUTE A WARRENTY, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PUPOSE"

Safety Data Sheet

Safety Data Sheet according to Regulation (EC) No. 1907/2006 (REACH)
Classification according to Regulation (EC) No. 1272/2008 [CLP]

SECTION 1: Identification of the substance/mixture and of the company/undertaking

Product identifier

Product code	15400054
Product name	TRYPSIN-EDTA 10X
Chemical Name	Not Applicable
REACH registration number	No registration number is given yet for this substance / substances in this mixture since the annual import quantity is less than one tonnage per annum or the transition period for its registration according to Article 23 of REACH has not yet expired.

Relevant identified uses of the substance or mixture and uses advised against

Relevant identified uses	CAUTION: For use as a raw material component in further manufacturing applications
Use Description Code	Not Applicable
Uses advised against	Not for consumer use.

Details of the supplier of the safety data sheet

Manufacturer / Supplier

LIFE TECHNOLOGIES EUROPE BV
KWARTSWEG 2
2665 NN BLEISWIJK
NETHERLANDS
31-(0)180 392 400
Email: MSDS@lifetech.com

Life Technologies Limited
3 Fountain Drive
Inchinnan Business Park
Paisley
PA4 9RF, UK
+44 (0)141 814 6100

24 hour Emergency Response for Hazardous Materials [or Dangerous Goods] Incident. Spill, Leak, Fire, Exposure, or Accident. Call CHEMTREC
Within the USA + Canada: 1-800-424-9300 and 1-703-527-3887
Outside the USA + Canada: 1-703-741-5970

Country Specific Emergency Number (if available):

CHEMTREC Ireland (Dublin)	+(353)-19014670 (Greeting Language: English and Irish)
CHEMTREC UK (London)	+(44)-870-8200418 (Greeting Language: English)

SECTION 2: Hazards identification

Classification of the substance or mixture

Classification according to Regulation (EC) No. 1272/2008 [CLP]

Physical hazards
Not Hazardous

Health hazards

Respiratory sensitiser	Category 1

Environmental hazards
Not Hazardous

Additional information
No information available

Label elements

Labelling according to Regulation (EC) No 1272/2008 [CLP]

Hazard pictograms

Signal Word
Danger

Hazard Statements
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled

Precautionary Statements

Prevention
P261 - Avoid breathing dust/fume/gas/mist/vapours/spray
P284 - In case of inadequate ventilation wear respiratory protection

Response
P342 + P311 - If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician
P304 + P340 - IF INHALED: Remove person to fresh air and keep comfortable for breathing

Storage
Not Applicable

Disposal
P501 - Dispose of contents/ container to an approved waste disposal plant

Other hazards
Not Applicable

SECTION 3: Composition/information on ingredients

Component	CAS No	EINECS-No.	Weight-%	REACH registration number	Classification according to Regulation (EC) No. 1272/2008 [CLP]
Trypsin 9002-07-7 (0.1-1.0%)	9002-07-7	232-650-8	0.1-1.0%	-	Eye Irrit. 2 - H319 STOT SE 3 - H335 Skin Irrit. 2 - H315 Resp. Sens. 1 - H334

SECTION 4: First aid measures

Description of first aid measures

Skin contact Wash off immediately with plenty of water for at least 15 minutes. Remove and wash contaminated clothing and gloves, including the inside, before re-use. Immediate medical attention is required.

Eye contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Immediate medical attention is required.

Ingestion Never give anything by mouth to an unconscious person. Do not induce vomiting without medical advice. Get medical attention if symptoms occur.

Inhalation Remove to fresh air. If not breathing, give artificial respiration. If symptoms persist, call a doctor.

Notes to Physician Treat symptomatically.

Most important symptoms and effects, both acute and delayed
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled

Indication of any immediate medical attention and special treatment needed
IF INHALED: Remove person to fresh air and keep comfortable for breathing. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.

SECTION 5: Firefighting measures

Extinguishing media

Suitable extinguishing media Water spray. Carbon dioxide (CO_2). Foam. Dry chemical.

Unsuitable extinguishing media No information available.

Special hazards arising from the substance or mixture
None known

Protective equipment and precautions for firefighters
Wear self-contained breathing apparatus and protective suit.

SECTION 6: Accidental release measures

Personal precautions, protective equipment and emergency procedures
Ensure adequate ventilation
Avoid contact with skin, eyes or clothing
Use personal protection equipment
See section 8 for more information

Environmental precautions
No special environmental precautions required. Avoid discharge into drains and waterways whenever possible.

Methods and material for containment and cleaning up
Soak up with inert absorbent material.

Reference to other sections
See section 8 for more information.

SECTION 7: Handling and storage

Precautions for safe handling
Always wear recommended Personal Protective Equipment. See section 8 for more information. Do not get in eyes, on skin, or on clothing. Do not ingest. If during normal use the material presents a respiratory hazard, use adequate ventilation and/or wear appropriate respirator.

Conditions for safe storage, including any incompatibilities
Keep in a dry, cool and well-ventilated place. Keep in properly labelled containers. Store in accordance with local regulations.

Specific end use(s)
CAUTION: For use as a raw material component in further manufacturing applications.

SECTION 8: Exposure controls/personal protection

Control parameters

Chemical Name	EU OEL (TWA)	EU OEL (STEL)	EU Skin Notation
Trypsin 9002-07-7	None	None	None

Chemical Name	Austria	Belgium (TWA)	Czech Republic
Trypsin 9002-07-7	None	None	None

Chemical Name	Denmark (TWA)	Finland OEL (TWA)	France OEL (VME)
Trypsin 9002-07-7	None	None	None

Chemical Name	Germany OEL (TWA)	Ireland (TWA)	Italy OEL (TWA)
Trypsin 9002-07-7	None	None	None

Chemical Name	Lithuania OEL (TWA)	Netherlands OEL (MAC)	Norway
Trypsin 9002-07-7	None	None	None

Chemical Name	Poland	Portugal	Spain OEL (TWA)
Trypsin 9002-07-7	None	None	None

Chemical Name	Sweden - Occupational Exposure Limits - TLVs (LLVs)	Switzerland	United Kingdom
Trypsin 9002-07-7	None	None	None

Engineering Measures Ensure adequate ventilation, especially in confined areas.

Exposure controls

Personal protection equipment

Respiratory protection In case of insufficient ventilation wear respirators and components tested and approved under appropriate government standards.

Hand protection Wear suitable gloves Glove material: Compatible chemical-resistant gloves.

Eye protection Tight sealing safety goggles.

Skin and Body Protection Wear laboratory coat for body protection.

Hygiene Measures Handle in accordance with good industrial hygiene and safety practice.

Environmental exposure controls
No special environmental precautions required.

SECTION 9: Physical and chemical properties

Information on basic physical and chemical properties

Appearance	liquid	
Odour	Mixture has not been tested	
Odour Threshold	Mixture has not been tested	
Molecular Weight	No data	
pH	No data	
Melting point / melting range	**°C** No data	**°F** No data
Boiling point / boiling range	**°C** No data	**°F** No data
Flash point	**°C** No data	**°F** No data
Autoignition Temperature	**°C** No data	**°F** No data
Decomposition temperature	**°C** No data	**°F** No data
Evaporation rate	No data	
Flammability (solid, gas)	Not Applicable	
Upper explosion limit	No data	
Lower explosion limit	No data	
Vapour Pressure	No data	
Vapour density	No data	
Relative density	No data	
Specific gravity	No data	
Solubility	Mixture has not been tested	
Partition coefficient: n-octanol/water	No data	
Viscosity	Mixture has not been tested	
Explosive properties	Mixture has not been tested	
Oxidising properties	Mixture has not been tested	

Other information
No data.

SECTION 10: Stability and reactivity

Reactivity	None known.
Chemical stability	Stable under normal conditions.
Possibility of hazardous reactions	Hazardous reaction has not been reported.
Conditions to avoid	None known.
Incompatible materials	No dangerous reaction known under conditions of normal use.
Hazardous decomposition products	No known hazardous decomposition products.

SECTION 11: Toxicological information

Information on toxicological effects

Chemical Name	Oral LD50	Dermal LD50	Inhalation LC50
Trypsin	> 5 g/kg (Rat)	No data available	No data available

Principal Routes of Exposure

Skin corrosion/irritation Data are conclusive but insufficient for classification

Serious eye damage/irritation Data are conclusive but insufficient for classification

Respiratory or skin sensitisation May cause sensitisation by inhalation

Specific target organ toxicity (STOT) – single exposure Data are conclusive but insufficient for classification

Specific target organ toxicity (STOT) – repeated exposure Data are conclusive but insufficient for classification

Carcinogenicity Data are conclusive but insufficient for classification

Germ cell mutagenicity Data are conclusive but insufficient for classification

Reproductive Toxicity Data are conclusive but insufficient for classification

Aspiration Hazard Data are conclusive but insufficient for classification

SECTION 12: Ecological information

Ecotoxicity
The environmental impact of this product has not been fully investigated.

Chemical Name	Toxicity to algae	Toxicity to daphnia and other aquatic invertebrates	Toxicity to fish	Microtox Data	log Pow
Trypsin	No data available	No data available	No data available	No data available	No data available

Persistence and degradability No information available.

Bioaccumulative potential No information available.

Results of PBT and vPvB assessment
No information available.

Other adverse effects
No information available.

SECTION 13: Disposal considerations

Waste treatment methods
The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique. Disposal of this product, its solutions or of any by-products, shall comply with the requirements of all applicable local, regional or national/federal regulations.

SECTION 14: Transport information

IATA / ADR / DOT-US / IMDG
Not regulated in the meaning of transport regulations

UN number	Not Applicable
UN proper shipping name	Not Applicable
Transport hazard class(es)	Not Applicable
Packing group	Not Applicable

Environmental hazards
Not Applicable

Special precautions for user
Not Applicable

Transport in bulk according to Annex II of MARPOL and the IBC Code
Not Applicable.

SECTION 15: Regulatory information

Safety, health and environmental regulations/legislation specific for the substance or mixture
None.

Substances of Very High Concern
None.

Substance subject to authorisation per REACH Annex XIV
None

Restricted substances under EC 1907/2006, Annex XVII
None.

Substances listed under Annex I of Regulation (EC) No 689/2008
None.

Restricted substances under Annex V of Regulation (EC) No 689/2008

None.

Substances under Regulation (EC) No 850/2004 of the European Parliament and of the Council of 29 April 2004 on persistent organic pollutants and amending Directive 79/117/EEC
None.

German Water hazard classes (Wassergefährdungsklassen)
Not classified.

Other International Inventories

Chemical Name	EINECS (European Union)	ELINCS (European List of Notified Chemical Substances)	ENCS (Japan)	PICCS (Philippines)
Trypsin	Listed	-	-	Listed

Chemical Name	AICS (Australia)	South Korea (KECL)	Canada (DSL)	NDSL
Trypsin	Listed	Listed	-	-

Chemical safety assessment
No Chemical safety assessment has been carried out.

SECTION 16: Other information

Reason for revision Update according to Commission Regulation (EU) No 830/2015
Revision number 4
Revision date 06-Aug-2019

References
• ECHA: http://echa.europa.eu/
• TOXNET: http://toxnet.nlm.nih.gov/
• eChemPortal: http://www.echemportal.org/
• LOLI database: https://www.chemadvisor.com/loli-database

Classification and procedure used to derive the classification for mixtures according to Regulation (EC) 1272/2008 [CLP]:

Respiratory sensitiser Category 1 Calculation method

"The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS SDS DOES NOT CONSTITUTE A WARRANTY, EXPRESSED OR IMPLIED,INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE"

gibco
by life technologies

AmnioMAX™ C-100 and AmnioMAX™ II Complete Media

Description

AmnioMAX™ products have been formulated and qualified for the *in vitro* propagation of primary cultures of human amniotic fluid cells and chorionic villus samples for use in prenatal diagnostic testing. AmnioMAX™ products have been optimized to maximize colony attachment, growth rates, pH stability, and to provide prolific metaphasic yield. AmnioMAX™ C-100 Complete Medium consists of an optimized basal medium, AmnioMAX™ C-100 Basal Medium, and supplement, AmnioMAX™ C-100 Supplement, containing an appropriate amount of antibiotics (gentamicin) and growth supplements to eliminate the need for further supplementation. AmnioMAX™ II Complete media is a second-generation formulation to improve cell morphology and provide cleaner cultures in a ready-to-use and convenient format which already containing antibiotics (gentamicin), L-glutamine and FBS. Every manufactured lot of AmnioMAX™ product is tested against rigorous standards to ensure clinical performance.

Product	Catalog No.	Amount	Storage	Shelf Life*
AmnioMAX™ C-100 Complete Medium, kit Kit contains:	12558-011	Kit		
AmnioMAX™ C-100 Basal Medium (1X), liquid	17001-082	90 mL	2°C to 8°C; Protect from light	—
AmnioMAX™ C-100 Supplement	12556-015	15 mL	–20°C to –5°C; Protect from light	—
AmnioMAX™ C-100 Basal Medium (1X), liquid	17001-082 17001-074	90 mL 450 mL	2°C to 8°C; Protect from light	16 months
AmnioMAX™ C-100 Supplement	12556-015 12556-023	15 mL 75 mL	–20°C to –5°C; Protect from light	16 months
AmnioMAX™ II Complete Medium	11269-016	100 mL	–20°C to –5°C; Protect from light	18 months

* Shelf Life duration is determined from Date of Manufacture. Do not use beyond labeled expiration date.

Intended Use

For *in vitro* diagnostic use.

Important Information

Do not use products if:

–Packaging has been compromised
–Product was received completely thawed
–AmnioMAX™ C-100 Basal or AmnioMAX™ II Complete Medium appears cloudy

Safety Information

For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare Media

AmnioMAX™ II Complete Medium is supplied frozen, ready to use upon thawing. Thaw at 2°C to 8°C, mix by gently swirling to ensure homogeneity. **Do not thaw** at 37°C. This may result in formation of a precipitate and should be avoided.

Note: AmnioMAX™ Media contain Fetal Bovine Serum (FBS); flocculent debris may develop upon thawing and storage.

- Thawed unopened AmnioMAX™ II Complete Medium can be stored in the dark at 2° to 8°C for up to two months within the labeled expiration date.
- Once opened, use AmnioMAX™ products within 7–10 days for maximal growth performance. Repeated warming/cooling and prolonged exposure to light should be avoided.
- **Do not use** beyond labeled expiration date.

Supplement Media

AmnioMAX™ II Complete Medium requires no further supplementation.

AmnioMAX™ C-100 Basal requires supplementation with AmnioMAX™ C-100 Supplement.

1. Aseptically add entire contents (15 mL) of AmnioMAX™ C-100 Supplement to 90 mL AmnioMAX™ C-100 Basal Medium before use.
2. Mix by gently swirling to ensure homogeneity.
3. Store in the dark at 2°C to 8°C until use.

Additional supplementation to AmnioMAX™ products is NOT recommended. **Note:** Addition of Fungizone® may be toxic.

Related Products

Product	Catalog No.
Lab Armor™ Beads	A12543
KaryoMAX® Colcemid™ Solution, liquid (10 µg/mL), in HBSS	15210
KaryoMAX® Colcemid™ Solution, liquid (10 µg/mL), in PBS	15212
KaryoMAX® Giemsa Stain Stock Solution	10092
Gurr Buffer Tablets (pH 6.8)	10582
Phytohemagglutinin (M Form)	10576-015
PB-MAX™ Karyotyping Medium	12557
MarrowMAX™ Bone Marrow Medium	12260

Each clinician/scientist must make an independent judgment on whether this medium is suitable for use in *In Vitro Diagnostic* applications conducted in their laboratory. Life Technologies does not guarantee the successful outcome of any diagnostic testing based solely on the use of GIBCO® medium. Life Technologies contribution to these procedures is simply at the step of providing a culture or handling medium for these procedures.

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

			
Caution, consult accompanying documents	*In vitro* diagnostic medical device	Sterilized using aseptic processing techniques	Protect from light
			
Use By:	Catalog number	Manufacturer	Batch Code
			
European Community	Consult instructions for use	Temperature Limitation	

Limited Use Label License

No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support
For further assistance, email **techsupport@lifetech.com**

life technologies™

CERTIFICATE OF REGISTRATION

This is to certify that

Thermo Fisher Scientific

Thermo Fisher Scientific
2215 Grand Avenue Parkway, Austin, Texas 78728 USA

operates a

Quality Management System

which complies with the requirements of

ISO 9001:2015

for the following scope of certification

The scope of this management system includes product design, validation and support services for the Gas Chromatography and Mass Spectrometry (GC/GCMS) product lines.

Certificate No.:	CERT-0113173	Original Certification Date:	March 31, 2009
File No.:	1605924	Certification Effective Date:	January 22, 2018
Issue Date:	January 29, 2018	Certificate Expiry Date:	January 21, 2021

Nicole Grantham
General Manager SAI Global Certification Services

ISO 9001

applied biosystems
by life technologies

GeneScan™ 500 LIZ® Size Standard

Catalog Number 4322682

Pub. no. 4363115 **Rev.** C

Contents†	Size	Storage conditions
LIZ® dye-labeled, single-stranded DNA fragments	2 X 200 µL (800 reactions‡)	Store up to one year at 2–8°C. Do not freeze.

† With the retirement of the ABI PRISM® 377 DNA Sequencer this kit no longer contains the gel loading buffer. If you need to continue to purchase the loading buffer, it is available as a separate part (Cat. no. 402055).

‡ 800 reactions when using the recommended loading amount of 0.5 µL.

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product description

GeneScan™ 500 LIZ® Size Standard is an internal lane size standard developed for use with Life Technologies fluorescence-based DNA electrophoresis systems. The use of an internal lane size standard during electrophoresis enables automated data analysis and precise DNA fragment size comparisons between electrophoresis runs. GeneScan™ 500 LIZ® Size Standard is designed for sizing DNA fragments in the 35–500 bp range and provides 16 single-stranded labeled fragments of 35, 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490, and 500 bases. Each of the DNA fragments is labeled with the LIZ® fluorophore, which results in a single peak when run under denaturing conditions.

Instructions for use

Sample preparation

1. Before use, mix the contents of each tube thoroughly and centrifuge briefly to collect the liquid at the bottom of the tube. Typical loading cocktails are as follows:

Component	Electrophoresis system		
	310	3130/3100 Series	3730 Series
Sample	0.5 µL	0.5 µL	0.5 µL
GeneScan™ 500 LIZ® Size Standard	0.5 µL	0.25 µL	0.5 µL
Hi-Di™ Formamide (Cat. no. 4311320)†	9.0 µL	9.25 µL	9.0 µL

† Not included in this kit.

Note: We highly recommend using the ratios of DNA sample (PCR product) and size standard presented in the table as a starting point only. Optimize the ratios if necessary, based on your experimental results. Also, see guidelines in the following section.

2. Heat the loading cocktail for 3 minutes at 95°C.
3. Immediately chill on ice for a few minutes and load samples.

Guidelines

Optimize your analysis based on the following information about use of this standard on capillary electrophoresis instruments:

- The 250 bp peak is sensitive to small temperature variations. The 250 bp fragment should not be used when defining the size standard in GeneMapper® Software.
- The 340 bp peak is subject to large temperature variations.
- Fragment analysis primer peaks can often interfere with the detection of the 35 bp peak.

Note: Discard any unused reagent that has been diluted in Hi-Di™ Formamide.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

For support visit **lifetechnologies.com/support** or email **techsupport@lifetech.com**

lifetechnologies.com

13 December 2012

molecular biology

Thermo SCIENTIFIC

Experience the PCR performance of your dreams

DreamTaq DNA Polymerase

Experience the PCR performance no conventional *Taq* enzyme can match with Thermo Scientific™ DreamTaq™ DNA Polymerase. This enhanced *Taq* DNA polymerase is designed to consistently and reliably deliver greater sensitivity, better yields, and longer amplicons. Reach the PCR performance of your dreams with Thermo Scientific™ DreamTaq™ reagents that offer:

- Robust amplification with minimal optimization
- Higher yields, sensitivity, and target length than conventional *Taq* enzymes
- Multiple formats for maximum flexibility and reliability
- Direct gel loading to simplify workflows

Robust amplification across different targets

DreamTaq DNA Polymerase outperforms conventional *Taq* enzymes, providing higher yields and amplifying longer amplicons (Figure 1). With DreamTaq DNA Polymerase, it's possible to achieve robust amplification of targets up to 6 kb from genomic DNA and up to 20 kb from viral DNA.

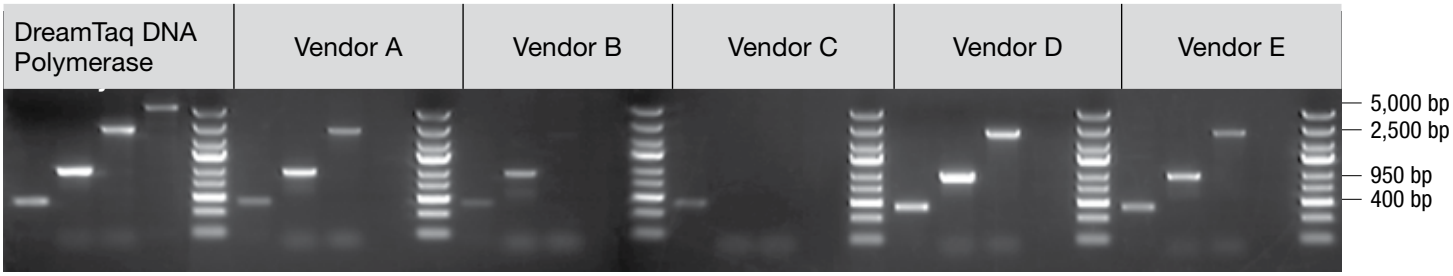


Figure 1. Robust amplification with DreamTaq DNA Polymerase.
DNA fragments of increasing length were amplified with DreamTaq DNA Polymerase and *Taq* DNA polymerase from different vendors according to manufacturers' recommendations. Only DreamTaq DNA Polymerase was able to amplify all fragments with high yields. DNA ladder: Thermo Scientific™ GeneRuler™ Express DNA Ladder.

ThermoFisher SCIENTIFIC

Thermo SCIENTIFIC

High sensitivity and yields

DreamTaq DNA Polymerase provides higher PCR sensitivity in comparison to conventional *Taq* enzymes. Robust amplification can be achieved even with low amounts of template DNA (Figure 2).

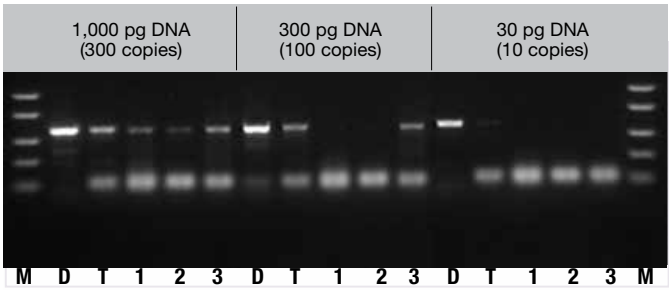


Figure 2. Superior yields with low amounts of DNA template. A 545 bp fragment from a human gene was amplified with DreamTaq DNA Polymerase and *Taq* DNA polymerases from other vendors. Reactions were performed according to manufacturers' recommendations using decreasing amounts of template DNA.
M: Thermo Scientific™ FastRuler™ Low-Range DNA Ladder
D: DreamTaq DNA Polymerase
T: Thermo Scientific™ *Taq* DNA Polymerase
1–3: *Taq* DNA polymerases from other vendors

Ready-to-load PCR products

DreamTaq DNA Polymerase is provided with both colorless and green reaction buffer. The Thermo Scientific™ DreamTaq™ Green Buffer allows direct loading of PCR products on gels and thus simplifies PCR workflows. The DreamTaq Green Buffer supports the same enhanced enzyme performance as the colorless buffer (Figure 3).

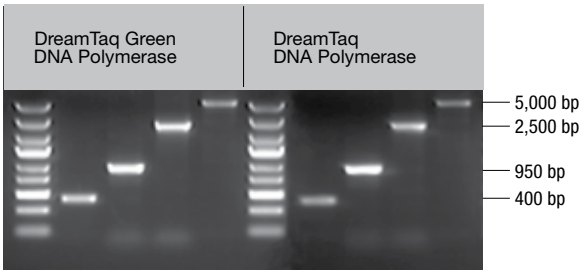


Figure 3. Equal PCR performance with green and colorless reaction buffer. Four DNA fragments were amplified with DreamTaq and Thermo Scientific™ DreamTaq™ Green DNA Polymerase with equal efficiency. DNA ladder: GeneRuler Express DNA Ladder.

Ordering information

Product	Size	Cat. No.
DreamTaq DNA Polymerase	200 U	EP0701
	500 U	EP0702
	5 x 500 U	EP0703
	20 x 500 U	EP0704
	10 x 500 U	EP0705
DreamTaq PCR Master Mix	200 x 50 µL rxns	K1071
	1,000 x 50 µL rxns	K1072
DreamTaq Green DNA Polymerase	200 U	EP0711
	500 U	EP0712
	5 x 500 U	EP0713
	20 x 500 U	EP0714
DreamTaq Green PCR Master Mix	200 x 50 µL rxns	K1081
	1,000 x 50 µL rxns	K1082

What is the DreamTaq Green Buffer?

The 10X DreamTaq Green Buffer includes a density reagent and two tracking dyes. One dye migrates with 3–5 kb DNA fragments and the other dye migrates faster than 10 bp DNA fragments in a 1% agarose gel (Figure 4). The colored buffer is compatible with downstream applications such as DNA sequencing, phosphorylation, ligation, and restriction digestion.

Figure 4. Reaction mixture with DreamTaq Green Buffer (A) prior to electrophoresis, (B) 5 min after electrophoresis, and (C) 15 min after electrophoresis.

Find out more at **thermofisher.com/dreamtaq**

ThermoFisher SCIENTIFIC

By Royal Charter

Certificate of Registration

QUALITY MANAGEMENT SYSTEM - ISO 9001:2015

This is to certify that:

Thermo Fisher Scientific Baltics
V. A.Graiciuno 8
Vilnius
LT-02241
Lithuania

Holds Certificate No: **FM 642793**

and operates a Quality Management System which complies with the requirements of ISO 9001:2015 for the following scope:

Design, development, manufacturing and sales of life science research products, including proteins, nucleic acids, nucleotides, antibodies, bio-sample preparation and cell separation reagents and associated kits, for research and in vitro diagnostics..

For and on behalf of BSI:

Andrew Launn, EMEA Systems Certification Director

Original Registration Date: 2016-03-25
Latest Revision Date: 2018-05-10

Effective Date: 2018-05-23
Expiry Date: 2021-05-22

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CERTIFICATE OF REGISTRATION

This is to certify that

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Refer to Attachment to Certificate of Registration dated May 02, 2014 for certified sites

operates a

Quality Management System

which complies with the requirements of

ISO 9001:2008

for the following scope of registration

Sales administration, customer service and support activities of analytical instruments, laboratory equipment, accessories, consumables and other related products

Certificate No.: **CERT-0063407**

Issue Date: **Nov 17, 2015**

Original Certification Date: **May 13, 2011**
Current Certification Date: **May 02, 2014**
Certificate Expiry Date: **May 01, 2017**

Chris Jouppi
President,
QMI-SAI Canada Limited

Heather Mahon
Acting Head of Head of Policy,
Risk and Certification

ISO 9001

ATTACHMENT TO

CERTIFICATE OF REGISTRATION

These sites are registered under Certificate No: **CERT-0063407** issued on **May 02, 2014**

Site	Effective Date
Thermo Fisher Scientific (Schweiz) AG Neuhofstrasse 11, 4153 Reinach | Switzerland	May 02, 2014
Thermo Electron Sweden AB Telefonvagen 30 2tr, 12626 Hagersten | Sweden	June 19, 2013
Thermo Electron Manufacturing Limited Stafford House, Boundary Way | HP2 7GEHemel Hempstead | United Kingdom	May 13, 2011
Thermo Electron S.a.s. Bld Sebastien Brant - BP 30188 | 67405 Illkirch Cedex | France	May 02, 2014
Thermo Electron S.a.s. 16 avenue du Québec - BP 30210 91941 Courtaboeuf Cedex | France	June 13, 2013
Thermo Electron S.a.s. 10 rue Duguay Trouin | 44807 Saint-Herblain | France	Aug 04, 2015
Thermo Fisher Scientific BVBA Clintonpark Keppekouter Ninovesteenweg 198 B-9320 Erembodegem-Aalst | Belgium	May 02, 2014
Thermo Electron A/S Stamholmen 193, 2650 Hvidovre | Denmark	June 19, 2013
Thermo Fisher Scientific wissenschaftliche Geraete GmbH Bürohaus Big Biz B Dresdner Strasse 89 | 1200 Wien Austria	May 02, 2014
Thermo Fisher Scientific S.L.U Avenida de la Vega 1, Edificio 1 Planta 4 28108 Alcobendas Spain	June 01, 2012
Thermo Fisher Scientific B.V. Takkebijsters 1 | 4817 Breda Netherlands	January 06,2012
Thermo Fisher Scientific SpA Strada Rivoltana, snc (km 4) | 20090 Rodano (MI) Italy	June 19, 2013
Thermo Fisher Scientific GmbH Im Steingrund 4-6 - 63303 Dreieich | Germany	June 19, 2013

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Thermo Electron S.a.s. 16 avenue du Québec - BP 30210 91941 Courtaboeuf Cedex | France	June 13, 2013
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Thermo Fisher Scientific GmbH Im Steingrund 4-6 - 63303 Dreieich | Germany	June 19, 2013

By Royal Charter

Certificate of Registration

QUALITY MANAGEMENT SYSTEM - ISO 13485:2016 & EN ISO 13485:2016

This is to certify that:

Thermo Fisher Scientific Baltics
V. A.Graiciuno 8
Vilnius LT-02241
Lithuania

Holds Certificate Number: MD 642790

and operates a Quality Management System which complies with the requirements of ISO 13485:2016 & EN ISO 13485:2016 for the following scope:

Design, development and manufacturing of life science products, including proteins, nucleic acids, nucleotides, antibodies and associated kits, for research and in vitro diagnostics, as well as manufacturing of the materials intended for ex-vivo separation of human cells and for cell-based clinical diagnostics and for therapeutic applications, including processes under aseptic conditions.

For and on behalf of BSI:

Stewart Brain, Head of Compliance & Risk - Medical Devices

Original Registration Date: 2016-02-15
Effective Date: 2018-05-23
Latest Revision Date: 2018-05-22
Expiry Date: 2021-05-22

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Certificate No: MD 642790

Location	Registered Activities
Thermo Fisher Scientific Baltics V. A.Graiciuno 8 Vilnius LT-02241 Lithuania	Design, development and manufacturing of life science products, including proteins, nucleic acids, nucleotides, antibodies and associated kits, for research and in vitro diagnostics, as well as manufacturing of the materials intended for ex-vivo separation of human cells and for cell-based clinical diagnostics and for therapeutic applications, including processes under aseptic conditions.

Original Registration Date: 2016-02-15

Effective Date: 2018-05-23

Latest Revision Date: 2018-05-22

Expiry Date: 2021-05-22

bsi.

By Royal Charter

Certificate of Registration

QUALITY MANAGEMENT SYSTEM - ISO 13485:2003

This is to certify that:

Life Technologies Corporation
Also Trading As: Invitrogen
3175 Staley Road
Grand Island
New York
14072
USA

Holds Certificate No: **FM 509223**

and operates a Quality Management System which complies with the requirements of ISO 13485:2003 for the following scope:

The design, development, manufacture and distribution of liquid and powder tissue culture media, sera, reagents and distribution of biochemicals.

The above activities are for cell culture research, industrial bioprocessing and related markets.

For and on behalf of BSI:

Reg Blake, VP Regulatory Affairs, BSI Group America Inc.

Original Registration Date: 01/16/2007 Effective Date: 11/06/2015 Expiry Date: 11/05/2018

CMDCAS Recognized Registrar

SCC Accredited CB-MS OCSM Accrédité CCN

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Certificate No: **FM 509223**

Location	Registered Activities
Life Technologies Corporation Also Trading As: Invitrogen 3175 Staley Road Grand Island New York 14072 USA	The design, development, manufacture and distribution of liquid and powder tissue culture media, sera, reagents and distribution of biochemicals. The above activities are for cell culture research, industrial bioprocessing and related markets.
Life Technologies Corporation Also Trading As: Invitrogen 1775 Baseline Road Grand Island New York 14072 USA	Product laboratory testing - Mycoplasma and Sales.

Original Registration Date: 01/16/2007 Effective Date: 11/06/2015 Expiry Date: 11/05/2018

By Royal Charter

Certificate of Registration

QUALITY MANAGEMENT SYSTEM - ISO 13485:2003 & EN ISO 13485:2012

This is to certify that:

Life Technologies Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley
PA4 9RF
United Kingdom

Holds Certificate Number: MD 507152

and operates a Quality Management System which complies with the requirements of ISO 13485:2003 & EN ISO 13485:2012 for the following scope:

The design, manufacture and distribution of In-Vitro Diagnostics and products for cell culture, molecular biology and microbiology.

For and on behalf of BSI:

Frank Lee, EMEA Compliance & Risk Director

Original Registration Date: 02/10/2006
Latest Revision Date: 24/09/2015

Effective Date: 02/10/2015
Expiry Date: 01/10/2018

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UKAS MANAGEMENT SYSTEMS 003

thermo scientific

PRODUCT INFORMATION

Exonuclease I

(Exo I)

Pub. No. MAN0012007
Rev. Date 29 November 2016 (Rev. B.00)

#_

Lot: _ **Expiry Date: _**

Store at -20 °C

Components	#EN0581	#EN0582
Exonuclease I (Exo I), 20 U/µL	4000 U	20000 U
10X Reaction Buffer	1 mL	5 × 1 mL

www.thermofisher.com

For Research Use Only. Not for use in diagnostic procedures.

Description

Exonuclease I (Exo I) degrades single-stranded DNA in a 3'→5' direction, releasing deoxyribonucleoside 5'-monophosphates in a stepwise manner and leaving 5'-terminal dinucleotides intact. It does not cleave DNA strands with terminal 3'-OH groups blocked by phosphoryl or acetyl groups (1).

Applications

- Primer removal from PCR mixtures:
  - prior to PCR product sequencing (2),
  - for one-tube “megaprimer” PCR mutagenesis (3).
- Removal of single-stranded DNA containing a 3'-hydroxyl terminus from nucleic acid mixtures.
- Assay for the presence of single-stranded DNA with a 3'-hydroxyl terminus (4).

Source

E.coli cells with a cloned *E.coli sbcB* gene.

Definition of Activity Unit

One unit of the enzyme catalyzes the release of 10 nmol of acid soluble nucleotides in 30 min at 37 °C. Enzyme activity is assayed in the following mixture: 67 mM glycine-KOH (pH 9.5), 6.7 mM $MgCl_2$, 1 mM DTT and 0.17 mg/mL single-stranded [^{3}H]-DNA.

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT and 50% (v/v) glycerol.

10X Reaction Buffer

670 mM glycine-KOH (pH 9.5 at 25 °C), 67 mM $MgCl_2$, 10 mM DTT.

Inhibition and Inactivation

- Inhibitors: 20% (w/v) PEG 8000 (5).
- Inactivated by heating at 80 °C for 15 min.

Note

The enzyme is not suitable for removing 3'-overhangs of dsDNA.

Protocol for PCR product clean-up prior to sequencing

The clean-up reaction removes unincorporated primers and degrades unincorporated nucleotides. The resulting PCR product is ready to use for sequencing without additional purification, e.g., using column purification kits.

1. Prepare the following reaction mixture:

PCR mixture (directly after completion of PCR)	5 µL
Exonuclease I	0.5 µL (10 U)
Thermo Scientific FastAP Thermosensitive Alkaline Phosphatase (#EF0651)	1 µL (1 U)

2. Mix well and incubate at 37 °C for 15 min.
3. Stop the reaction by heating the mixture at 85 °C for 15 min.

Note

- Up to 5 µL of purified PCR products can be used directly for DNA sequencing without further purification.
- For reliable sequencing results there should not be nonspecific PCR products.
- The protocol may be applied for clean-up of PCR products, generated by any thermophilic DNA polymerase or polymerase mix.
- The procedure is not recommended for downstream cloning applications.

(continued on back page)

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable degradation was observed after incubation of supercoiled plasmid DNA with Exonuclease I.

Single-stranded Endodeoxyribonuclease Assay

No detectable degradation was observed after incubation of circular single-stranded DNA with enzyme.

Double-stranded Exodeoxyribonuclease Assay

No degradation degradation was observed after Incubation of double-stranded DNA fragments with enzyme.

Ribonuclease Assay

No detectable degradation was observed after incubation of [3H]-RNA with Exonuclease I.

Quality authorized by: Jurgita Zilinskiene

Reference

1. Lehman, I.R., Nussbaum A.L., The deoxyribonucleases of *Escherichia coli*. V. On the specificity of exonuclease I (phosphodiesterase), J. Biol. Chem., 239, 2628-2636, 1964.
2. Werle, E., et al., Convenient single-step, one tube purification of PCR products for direct sequencing, Nucleic Acids Res., 22, 4354-4355, 1994.
3. Nabavi S., Nazar R.N., Simplified one tube “megaprimer” polymerase chain reaction mutagenesis, Anal Biochem., 2, 346-348, 2005.
4. Rosamond, J., et al., Modulation of the action of the recBC enzyme of *Escherichia coli* K-12 by Ca^{2+}, J. Biol. Chem., 254, 8646-8652, 1979.
5. Sasaki, Y., Miyoshi, D. and Sugimoto, N., Regulation of DN nucleases by molecular crowding., Nucleic Acids Res., 35, 4086-4093, 2007.

NOTICE TO PURCHASER:

The purchase of this product allows the purchaser to use it for preparing amplified DNA fragments under a license from GE Healthcare of U.S. Patent Nos. 5,741,676 and 5,756,285 and other foreign patents.

LIMITED USE LABEL LICENSE: Internal Research and Development Use Only.

The purchase of this product conveys to the buyer the limited, non-exclusive, non-transferable right (without the right to resell, repackage, or further sublicense) to use this product for internal research and development purposes. No other license is granted to the buyer whether expressly, by implication, by estoppel or otherwise. In particular, the purchase of the product does not include or carry any right or license to use, develop, or otherwise exploit this product commercially and no rights are conveyed to the buyer to use the product or components of the product for purposes including but not limited to provision of services to a third party, generation of commercial databases or clinical diagnostics. This product is sold pursuant to authorization from Thermo Fisher Scientific and Thermo Fisher Scientific reserves all other rights. For information on purchasing a license for uses other than internal research and development purposes, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies Inc., 5791 Van Allen Way, Carlsbad, California 92008.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermofisher.com for Material Safety Data Sheet of the product.

thermo scientific

PRODUCT INFORMATION

Thermo Scientific DreamTaq Green DNA Polymerase

Pub. No. MAN0012040
Rev. Date 23 December 2016 (Rev. B.00)

Lot: __ **Expiry Date:** __

Store at -20°C

Ordering Information

Component	DreamTaq DNA Polymerase, 5 U/µL	10X DreamTaq Green Buffer*
#EP0711	200 U	1.25 mL
#EP0712	500 U	2 × 1.25 mL
#EP0713	5 × 500 U	10 × 1.25 mL
#EP0714	20 × 500 U	40 × 1.25 mL

* includes 20 mM $MgCl_2$

www.thermofisher.com

For Research Use Only. Not for use in diagnostic procedures.

Description

Thermo Scientific™ DreamTaq™ Green DNA Polymerase is a combination of DreamTaq DNA Polymerase and 10X DreamTaq Green Buffer. DreamTaq DNA Polymerase is an enhanced Taq DNA Polymerase optimized for high throughput PCR applications. It ensures higher sensitivity, longer PCR products and higher yields compared to conventional Taq DNA polymerase. DreamTaq Green DNA Polymerase incorporates modified nucleotides, but is inhibited by dUTP.

The 10X DreamTaq Green Buffer includes a density reagent and two tracking dyes for direct loading of PCR products on a gel. The colored buffer does not interfere with PCR performance and is compatible with downstream applications such as DNA sequencing, phosphorylation, ligation and restriction digestion. For applications that require PCR product analysis by absorbance or fluorescence excitation, we recommend using the colorless 10X DreamTaq Buffer (#B65) or purifying the PCR product prior to analysis.

Features

- Save time – go directly from PCR to gel electrophoresis.
- High yields of PCR products with minimal optimization.
- Higher sensitivity compared to conventional *Taq* DNA Polymerase.
- Amplification of long targets (up to 6 kb from genomic DNA, up to 20 kb from viral DNA).
- Robust amplification of difficult templates.

Applications

- Routine PCR amplification of DNA fragments up to 6 kb from genomic DNA and up to 20 kb from viral DNA.
- RT-PCR.
- Genotyping.
- Generation of PCR products for TA cloning.

Concentration

5 U/µL

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 min at 74 °C.

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 8.0), 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5% (v/v) Nonidet P40, 0.5% (v/v) Tween 20 and 50% (v/v) glycerol.

10X DreamTaq Green Buffer

A proprietary formulation which, in addition to the PCR buffer components, includes a density reagent and two tracking dyes for direct loading of PCR products on a gel. The 10X DreamTaq Green Buffer contains KCl and $(NH_4)_2SO_4$ at a ratio optimized for robust performance in PCR and includes $MgCl_2$ at a concentration of 20 mM.

Inhibition and Inactivation

- Inhibitors: ionic detergents (deoxycholate, sarkosyl and SDS) at concentrations higher than 0.06, 0.02 and 0.01%, respectively.
- Inactivated by phenol/chloroform extraction.

PROTOCOL

To set up parallel reactions and to minimize the possibility of pipetting errors, prepare a PCR master mix by mixing water, buffer, dNTPs, primers and DreamTaq DNA Polymerase. Prepare enaugh master mix for the number of reactions plus one extra. Aliquot the master mix into individual PCR tubes and then add template DNA.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 50 µL reaction:

10X DreamTaq Green Buffer*	5 µL
dNTP Mix, 2 mM each (#R0241)	5 µL (0.2 mM of each)
Forward primer	0.1-1.0 µM
Reverse primer	0.1-1.0 µM
Template DNA	10 pg - 1 µg
DreamTaq DNA Polymerase	1.25 U
Water, nuclease-free (#R0581)	to 50 µL
Total volume	50 µL

*10X DreamTaq Green Buffer contains 20 mm $MgCl_2$, which is optimal for most applications. If additional optimization is required, 25 mm $MgCl_2$ (#R0971) can be added to the master mix. The volume of water should be reduced accordingly.

Volumes of 25 mM $MgCl_2$, required for specific final $MgCl_2$ concentration:

Final concentration of $MgCl_2$	2 mM	2.5 mM	3 mM	4 mM
Volume of 25 mM $MgCl_2$ to be added for 50 µL reaction	0 µL	1 µL	2 µL	4 µL

3. Gently vortex the samples and spin down.
4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µL of mineral oil.
5. Place the reactions in a thermal cycler. Perform PCR using recommended thermal cycling conditions:

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	95	1-3 min	1
Denaturation	95	30 s	25-40
Annealing	Tm-5	30 s	
Extension*	72	1 min	
Final Extension	72	5-15 min	1

* The recommended extension step is 1 min for PCR products up to 2 kb. For longer products, the extension time should be prolonged by 1 min/kb.

GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Use PCR-certified reagents, including high quality water (e.g., Water, nuclease-free, #R0581).
- Always perform “no template control” (NTC) reactions to check for contamination.

DreamTaq DNA Polymerase does not incorporate dUTP, therefore it is not possible to perform carryover contamination prevention with UDG. For this application we recommend using *Taq* DNA Polymerase (#EP0401) or Thermo Scientific™ DreamTaq™ Hot Start DNA polymerase (#EP1711).

GUIDELINES FOR PRIMER DESIGN

Use primer design software or follow general recommendations for PCR primer design as outlined below:

- PCR primers are generally 15-30 nucleotides long.
- Optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.
- Avoid placing more than three G or C nucleotides at the 3’-end to lower the risk of non-specific priming.
- If possible, the primer should terminate with a G or C at the 3’-end.
- Avoid self-complementary primer regions, complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementary between primers and template DNA.
- When designing degenerate primers, place at least 3 conserved nucleotides at the 3’-end.

- When introducing restriction enzyme sites into primers, refer to the table "Reaction conditions for FastDigest enzymes" located on www.thermofisher.com/fastdigest to determine the number of extra bases required for efficient cleavage.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.

Estimation of primer melting temperature

For primers containing less than 25 nucleotides, the approx. melting temperature (Tm) can be calculated using the following equation:

$$Tm = 4 (G + C) + 2 (A + T),$$

where G, C, A, T represent the number of respective nucleotides in the primer.

If the primer contains more than 25 nucleotides specialized computer programs are recommended to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA

Optimal amounts of template DNA for a 50 µL reaction volume are 0.01-1 ng for both plasmid and phage DNA, and 0.1-1 µg for genomic DNA. Higher amounts of template increase the risk of generation of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.

All routine DNA purification methods are suitable for template preparation e.g., Thermo Scientific™ GeneJET™ Genomic DNA Purification Kit (#K0721) or Thermo Scientific™ GeneJET™ Plasmid Miniprep Kit (#K0502). Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, can inhibit DNA polymerases. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol normally removes trace contaminants from DNA samples.

$MgCl_2$ concentration

DreamTaq Green DNA Polymerase is provided with an optimized 10X DreamTaq Green Buffer which includes $MgCl_2$ at a concentration of 20 mM. A final $MgCl_2$ concentration of 2 mM is generally ideal for PCR. The $MgCl_2$ concentration can be further increased up to 4 mM by the addition of 25 mM $MgCl_2$ (#R0971).

If the DNA samples contain EDTA or other metal chelators, the Mg^{2+} ion concentration in the PCR mixture should be increased accordingly (1 molecule of EDTA binds 1 Mg^{2+}).

dNTPs

The recommended final concentration of each dNTP is 0.2 mM. In certain PCR applications, higher dNTP concentrations may be necessary. It is essential to have equal concentrations of all four nucleotides (dATP, dCTP, dGTP and dTTP) present in the reaction mixture.

To obtain a 0.2 mM concentration of each dNTP in the PCR mixture, please refer to the table below:

Volume of PCR mixture	dNTP Mix, 2 mM each (#R0241)	dNTP Mix, 10 mM each (#R0191)	dNTP Mix, 25 mM each (#R1121)
50 µL	5 µL	1 µL	0.4 µL
25 µL	2.5 µL	0.5 µL	0.2 µL
20 µL	2 µL	0.4 µL	0.16 µL

Primers

The recommended concentration range of the PCR primers is 0.1-1 µM. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products.

For degenerate primers and primers used for long PCR, we recommend higher primer concentrations in the range of 0.3-1 µM.

CYCLING PARAMETERS

Initial DNA denaturation

It is essential to completely denature the template DNA at the beginning of the PCR run to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 95 °C is sufficient. For GC-rich templates this step should be prolonged up to 10 min. If a longer initial denaturation step is required, or if the DNA is denatured at a higher temperature, DreamTaq DNA Polymerase should be added after the initial denaturation step to avoid a decrease in its activity.

Denaturation

A DNA denaturation time of 30 seconds per cycle at 95°C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3-4 min. DNA denaturation can also be enhanced by the addition of either 10-15% glycerol, 10% DMSO, 5% formamide or 1-1.5 M betaine. The melting temperature of the primer-template complex decreases significantly in the presence of these reagents. Therefore, the annealing temperature has to be adjusted accordingly.

In addition, 10% DMSO and 5% formamide inhibit DNA polymerases by 50%. Thus, the amount of the enzyme in the reaction should be increased if these additives are used.

Primer annealing

The annealing temperature should be 5 °C lower than the melting temperature (Tm) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2 °C increments. When additives, which change the melting temperature of the primer-template complex, are used (glycerol, DMSO, formamide and betaine) the annealing temperature must also be adjusted.

Extension

The optimal extension temperature for DreamTaq DNA Polymerase is 70-75 °C. The recommended extension step is 1 min at 72°C for PCR products up to 2 kb. For longer products, the extension time should be prolonged by 1 min/kb. For amplification of templates >6 kb a reduction of the extension temperature to 68 °C is recommended to avoid enzyme inactivation during prolonged extension times.

Number of cycles

The number of cycles may vary depending on the amount of template DNA in the PCR mixture and the expected PCR product yield.

If less than 10 copies of the template are present in the reaction, about 40 cycles are required. For higher template amounts, 25-35 cycles are sufficient.

Final extension

After the last cycle, it is recommended to incubate the PCR mixture at 72 °C for additional 5-15 min to fill-in any possible incomplete reaction products. If the PCR product will be cloned into TA vectors (for instance, using Thermo Scientific™ InsTAclone™ PCR Cloning Kit (#K1213)), the final extension step may be prolonged to 30 min to ensure the complete 3'-dA tailing of the PCR product. If the PCR product will be used for cloning using Thermo Scientific™ CloneJET™ PCR Cloning Kit (#K1231), the final extension step can be omitted.

Troubleshooting

For troubleshooting please visit www.thermofisher.com

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No conversion of covalently closed circular DNA to nicked DNA was detected.

Exodeoxyribonuclease Assay

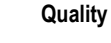
No degradation of DNA was observed after incubation of DNA fragments with DreamTaq Green DNA Polymerase.

Ribonuclease Assay

No contaminating RNase activity was detected.

Functional Assay

DreamTaq DNA Polymerase was tested for amplification of 956 bp single copy gene from human genomic DNA.

Quality authorized by:  Jurgita Žilinskienė

thermo scientific

PRODUCT INFORMATION

Thermo Scientific FastAP Thermosensitive Alkaline Phosphatase

Pub. No. MAN0012876
Rev. Date 29 November 2016 (Rev. B.00)

#__

Lot: __ Expiry Date: _

Store at -20 °C

Components	#EF0651	#EF0652	#EF0654
FastAP Thermosensitive Alkaline Phosphatase, 1 U/µL	1000 U for 1000 reactions	5 × 1000 U for 5000 reactions	300 U for 300 reactions
10X FastAP Buffer	2 × 1.5 mL	10 × 1.5 mL	1.5 mL

BSA included

www.thermofisher.com

For Research Use Only. Not for use in diagnostic procedures.

Description

Thermo Scientific™ FastAP™ Thermosensitive Alkaline Phosphatase catalyzes the release of 5'- and 3'- phosphate groups from DNA, RNA and nucleotides. This enzyme also removes phosphate groups from proteins. FastAP is a novel alkaline phosphatase, which is active in all Thermo Scientific restriction enzyme buffers as well as in PCR buffers. It dephosphorylates all types of DNA ends in 10 min at 37 °C. The enzyme is inactivated in 5 min at 75 °C. Therefore, removal of alkaline phosphatase is not required prior to ligation.

Applications

- Dephosphorylation of cloning vector DNA to prevent recircularization during ligation.
- Simultaneous digestion and dephosphorylation of vector DNA.
- PCR product clean-up: nucleotide degradation prior to sequencing of PCR product.
- Dephosphorylation of nucleic acid 5’-termini prior to labeling with T4 Polynucleotide Kinase.
- Other applications where dephosphorylation of DNA and RNA substrates is necessary.
- Protein dephosphorylation.

Source

E.coli cells with a cloned bacterial AP gene.

Definition of Activity Unit

One unit is the amount of the enzyme required to dephosphorylate 5'-termini of 1 µg of linearized pUC57 DNA in 10 min at 37 °C in FastAP buffer.

Storage Buffer

The enzyme is supplied in:
20 mM HEPES-NaOH (pH 7.4), 1 mM $MgCl_2$,
0.1 mM $ZnCl_2$, 0.1% (v/v) Triton X-100 and
50% (v/v) glycerol.

10X FastAP Buffer

100 mM Tris-HCl (pH 8.0 at 37 °C), 50 mM $MgCl_2$,
1 M KCl, 0.2% Triton X-100 and 1 mg/mL BSA.

Inhibition and Inactivation

- Inhibitors: metal chelators.
- Inactivated by heating at 75 °C for 5 min.

Note

- Binding of FastAP Thermosensitive Alkaline Phosphatase to DNA may result in a band shift in agarose gels. To avoid this, incubate samples with 6X Loading Dye & SDS Solution (#R1151) at 65 °C for 10 min and chill on ice prior to electrophoresis.
- FastAP Thermosensitive Alkaline Phosphatase is active in all restriction enzyme buffers and may be added directly to digested DNA. Heat inactivation of the restriction enzyme before dephosphorylation reaction is not necessary.

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable degradation was observed after incubation of supercoiled plasmid DNA with FastAP Thermosensitive Alkaline Phosphatase.

Ribonuclease Assay

No detectable degradation was observed after incubation of [3H]-RNA with FastAP Thermosensitive Alkaline Phosphatase.

Labeled Oligonucleotide (LO) Assay

No detectable degradation after incubation of single-stranded or double-stranded radiolabeled oligonucleotides with FastAP Thermosensitive Alkaline Phosphatase.

Quality authorized by:  Jurgita Zilinskiene

(continued on back page)

Protocol for fast simultaneous plasmid vector linearization and dephosphorylation

1. Prepare the following reaction mixture containing:

Plasmid DNA	1 µg
10X Thermo Scientific FastDigest Buffer	2 µL
FastDigest Restriction Enzyme	1 µL
FastAP Thermosensitive Alkaline Phosphatase	1 µL
Water, nuclease-free (#R0581)	to 20 µL
Total volume	20 µL

2. Mix thoroughly, spin briefly and incubate at 37 °C for 10 min.
3. Stop reactions by heating at 65 °C for 15 min or at 80 °C for 20 min (if restriction enzyme is not inactivated at 65 °C).

Note

For FastDigest SphI (PaeI) (#FD0604), simultaneous digestion and dephosphorylation is not recommended. Perform digestion, spin column purification and then dephosphorylation.

Protocol for nucleic acid dephosphorylation

This protocol is suitable for removal of 3’ and 5’ -phosphate groups from DNA and RNA.

1. Prepare the following reaction mixture:

Linear DNA (~3 kb plasmid)	1 µg (~1 pmol termini)
10X reaction buffer for AP used in reaction	2 µL
FastAP Thermosensitive Alkaline Phosphatase	1 µL (1 U)
Water, nuclease-free (#R0581)	to 20 µL
Total volume	20 µL

2. Mix thoroughly, spin briefly and incubate 10 min at 37 °C.
3. Stop reaction by heating for 5 min at 75 °C.

Note

For efficient dephosphorylation plasmid DNA should be free of RNA and genomic DNA.

Protocol for dephosphorylation of proteins

Reaction mixture:
1X FastAP reaction buffer, 0.1-0.2 mg/mL of phosphoprotein, 10 U of FastAP Thermosensitive Alkaline Phosphatase. Incubate at 37 °C for 1 h.
For example:
If you are doing a 20 µL reaction setup you need 2 µL 10X FastAP buffer, 2-4 µg of protein (to be in the range of 0.1-0.2 mg/mL) and 10 U of FastAP Thermosensitive Alkaline Phosphatase (1 U/µL).

Note

- The reaction can be stopped by addition of a final concentration of 50 mM EDTA (#R1021) or by addition of a final concentration of 10 mM sodium orthovanadate (Na_3VO_4).
- The optimal incubation time and the enzyme concentration must be determined experimentally for each substrate.

Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific DreamTaq Hot Start Green DNA Polymerase

Pub. No. MAN0015973
Rev. Date 02 August 2016 (Rev. A.00)

#__

Lot: __ Expiry Date: __

Ordering Information

Catalog No.	DreamTaq Hot Start DNA Polymerase, 5 U/µL	10X DreamTaq Green Buffer*
EP1711	200 U	1.25 mL
EP1712	500 U	2 × 1.25 mL
EP1713	2500 U	10 × 1.25 mL
EP1714	4 × 2500 U	40 × 1.25 mL

* includes 20 mM $MgCl_2$

Store at –20°C

www.thermofisher.com

For Research Use Only. Not for use in diagnostic procedures.

DESCRIPTION

Thermo Scientific™ DreamTaq™ Green DNA Polymerase is a combination of DreamTaq™ Hot Start DNA Polymerase and 10X DreamTaq Green Buffer. DreamTaq™ Hot Start DNA Polymerase is an enhanced hot start *Taq* DNA polymerase optimized for most PCR applications. It ensures higher sensitivity, specificity, and yields compared to conventional hot start *Taq* DNA polymerase. It is capable of amplifying long amplicons such as 6 kb genomic DNA and 20 kb λ DNA.

DreamTaq Hot Start DNA Polymerase combines *Taq* DNA polymerase and a specific antibody that inhibits the DNA polymerase activity at ambient temperatures, thus preventing the amplification of non-specific products. At polymerization temperatures, the antibody molecule is released, rendering the polymerase fully active.

DreamTaq Hot Start DNA Polymerase uses the same reaction set-up and cycling conditions as conventional *Taq* DNA polymerases, but the antibody-based hot start allows the reactions to be set up at room temperature. Because the enzyme is supplied with the optimized DreamTaq buffer, which includes 20 mM $MgCl_2$, extensive optimization of reaction conditions is not required.

DreamTaq Hot Start DNA Polymerase generates PCR products with 3'-dA overhangs. The enzyme tolerates dUTP and can incorporate modified nucleotides.

FEATURES

- High specificity due to antibody based hot start
- Save time – go directly from PCR to gel electrophoresis
- Robust amplification with minimal optimization
- High yields of PCR products
- Higher sensitivity compared to conventional hot start *Taq* DNA polymerases
- Amplification of long targets up to 6 kb from genomic DNA and up to 20 kb from viral DNA
- Generates 3'-dA overhangs
- Incorporates dUTP and modified nucleotides

APPLICATIONS

- Routine PCR amplification of DNA fragments up to 6 kb from genomic DNA and up to 20 kb from viral DNA
- RT-PCR
- Genotyping
- Generation of PCR products for TA cloning

CONCENTRATION

5 U/µL

DEFINITION OF ACTIVITY UNIT

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 minutes at 74°C.

10X DREAMTAQ GREEN BUFFER

DreamTaq Green Buffer is a proprietary formulation, which contains KCl and $(NH_4)_2SO_4$ at a ratio optimized for robust performance of DreamTaq Hot Start DNA Polymerase in PCR. The buffer includes $MgCl_2$ at a concentration of 20 mM.

The 10X DreamTaq Green Buffer includes a density reagent and two tracking dyes for direct loading of PCR products on a gel. The colored buffer does not interfere with PCR performance and is compatible with downstream applications such as DNA sequencing, ligation, and restriction digestion. For applications that require PCR product analysis by absorbance or fluorescence excitation, we recommend using the colorless 10X DreamTaq Buffer (#B65) or purifying the PCR product prior to analysis.

INHIBITION AND INACTIVATION

- Inhibitors: ionic detergents (deoxycholate, sarkosyl and SDS) at concentrations higher than 0.06, 0.02, and 0.01%, respectively.
- Inactivated by phenol/chloroform extraction.

PROTOCOL

To set up parallel reactions and to minimize the possibility of pipetting errors, prepare a PCR master mix by mixing water, buffer, dNTPs, primers, and DreamTaq Hot Start DNA Polymerase. Prepare sufficient master mix for the number of reactions plus one extra. Aliquot the master mix into individual PCR tubes, then add template DNA.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. For each 50-µL reaction, add the following components into a separate thin-walled PCR tube:

10X DreamTaq Green Buffer*	5 µL
dNTP Mix, 2 mM each (#R0241)	5 µL (0.2 mM of each)
Forward primer	0.1–1.0 µM
Reverse primer	0.1–1.0 µM
Template DNA	10 pg–1 µg
DreamTaq Hot Start DNA Polymerase	1.25 U
Water, nuclease-free (#R0581)	to 50 µL
Total volume	50 µL

*10X DreamTaq Green Buffer contains 20 mM $MgCl_2$, which is optimal for most applications. If further optimization is required, additional $MgCl_2$ can be added to the master mix. The volume of water should be reduced accordingly.

Volume of 25 mM $MgCl_2$ (#R0971) required for specific final $MgCl_2$ concentration:

Final concentration of $MgCl_2$	2 mM	2.5 mM	3 mM	4 mM
Volume of 25 mM $MgCl_2$ to add to 50-µL reaction	0 µL	1 µL	2 µL	4 µL

3. Gently vortex the samples and briefly centrifuge.
4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µL of mineral oil.
5. Place the reactions in a thermal cycler. Perform PCR using the recommended thermal cycling conditions outlined below:

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	95	1–3 min	1
Denaturation	95	30 s	25–40
Annealing	Tm	30 s	
Extension*	72	1 min	
Final Extension	72	5–15 min	1

* The recommended extension step is 1 minute for PCR products up to 2 kb. For longer products, the extension time should be prolonged by 1 minute/kb.

GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. Follow the general recommendations below to lower the risk of contamination.

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Use PCR-certified reagents, including high quality water (e.g., Water, nuclease-free, #R0581).
- Always perform "no template control" (NTC) reactions to check for contamination.

DreamTaq Hot Start DNA Polymerase incorporates dUTP; therefore, you can control carry-over contamination using Uracil-DNA Glycosylase (#EN0361).

GUIDELINES FOR PRIMER DESIGN

Use special design software or follow the general recommendations for PCR primer design as outlined below to design optimal primers:

- Use PCR primers that are 15–30 nucleotides long.
- Optimal GC content of the primer is 40–60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.

(continued on reverse page)

- Avoid placing more than three G or C nucleotides at the 3'-end to lower the risk of non-specific priming.
- If possible, the primer should terminate with a G or C at the 3'-end.
- Avoid self-complementary primer regions, and complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementarity between primers and template DNA.
- When designing degenerate primers, place at least 3 conserved nucleotides at the 3'-end.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.

ESTIMATION OF PRIMER MELTING TEMPERATURE

For primers containing less than 25 nucleotides, the approximate melting temperature (Tm) can be calculated using the following equation:

$$Tm = 4 (G + C) + 2 (A + T),$$

where G, C, A, T represent the number of respective nucleotides in the primer.

If the primer contains more than 25 nucleotides, we recommend using specialized computer programs to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA

Optimal amount of template DNA for a 50-µL reaction volume is 1 pg–1 ng for both plasmid and phage DNA, and 100 pg–1 µg for genomic DNA. Higher amounts of template increase the risk of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.

All routine DNA purification methods are suitable for template preparation; e.g., Thermo Scientific™ GeneJET™ Genomic DNA Purification Kit (#K0721) or GeneJET Plasmid Miniprep Kit (#K0502). Trace amounts of certain agents used for DNA purification, such as phenol, EDTA, and proteinase K, can inhibit DNA polymerases. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol normally removes trace contaminants from DNA samples.

$MgCl_2$ concentration

DreamTaq Hot Start DNA Polymerase is provided with an optimized 10X DreamTaq Green Buffer, which includes $MgCl_2$ at a concentration of 20 mM. A final $MgCl_2$ concentration of 2 mM is generally ideal for PCR. The $MgCl_2$ concentration can be further increased up to 4 mM by the addition of 25 mM $MgCl_2$ (#R0971).

If the DNA samples contain EDTA or other metal chelators, the Mg^{2+} ion concentration in the PCR mixture should be increased accordingly (1 molecule of EDTA binds 1 Mg^{2+}).

dNTPs

The recommended final concentration of each dNTP is 0.2 mM. In certain PCR applications, higher dNTP concentrations may be necessary. It is essential to have equal concentrations of all four nucleotides (dATP, dCTP, dGTP, and dTTP) in the reaction mixture.

To obtain a 0.2 mM concentration of each dNTP in the PCR mixture, refer to the table below.

Volume of PCR mixture	dNTP Mix, 2 mM each (#R0241)	dNTP Mix, 10 mM each (#R0191)	dNTP Mix, 25 mM each (#R1121)
50 µL	5 µL	1 µL	0.4 µL
25 µL	2.5 µL	0.5 µL	0.2 µL
20 µL	2 µL	0.4 µL	0.16 µL

Use 200 µM of each dNTP. dUTP or dITP can be added up to 200 µM. For longer amplicons, a lower dUTP concentration (20–100 µM) may be required for high yields.

Primers

The recommended concentration range of the PCR primers is 0.1–1 µM. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products.

For degenerate primers and primers used for long PCR, we recommend higher primer concentrations in the range of 0.3–1 µM.

CYCLING PARAMETERS

Initial DNA denaturation and enzyme activation

DreamTaq Hot Start DNA polymerase is inactive at room temperature during the reaction set up and is activated during the 1–3 minute initial denaturation/enzyme activation step.

It is essential to completely denature the template DNA at the beginning of the PCR run to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 60% or less, an initial 1–3 minute denaturation at 95°C is sufficient. For GC-rich templates this step can be prolonged.

Denaturation

A DNA denaturation time of 30 seconds per cycle at 95°C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3–4 minutes. DNA denaturation can also be enhanced by the addition of 5–10% glycerol, 5% DMSO, 1% formamide, or 1–1.5 M betaine. The melting temperature of the primer-template complex decreases significantly in the presence of these reagents. Therefore, the annealing temperature has to be adjusted accordingly.

Note that higher than 10% DMSO or 5% formamide in the reaction mix inhibits DNA polymerases. Therefore, it may be necessary to increase the amount of the enzyme in the reaction if these additives are used.

Primer annealing

The annealing temperature should be equal to the melting temperature (Tm) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1–2°C increments. When additives that change the melting temperature of the primer-template complex are used (glycerol, DMSO, formamide and betaine), the annealing temperature must also be adjusted.

Extension

The optimal extension temperature for DreamTaq Hot Start DNA Polymerase is 70–75°C. The recommended extension step is 1 minute at 72°C for PCR products up to 2 kb. For longer products, the extension time should be increased by 1 minute/kb. For amplification of templates >6 kb, we recommend reducing the extension temperature to 68°C.

Number of cycles

The number of cycles may vary depending on the amount of template DNA in the PCR mixture and the expected PCR product yield.

If less than 10 copies of the template is present in the reaction, about 40 cycles are required. For higher template amounts, 25–35 cycles are sufficient.

Final extension

After the last cycle, we recommend incubating the PCR mixture at 72°C for an additional 5–15 minutes to fill in any possible incomplete reaction products. If the PCR product will be cloned into TA vectors such as the Thermo Scientific™ InsTAclone™ PCR Cloning Kit (#K1213), the final extension step may be prolonged to 15 minutes to ensure the complete 3'-dA tailing of the PCR product. If the PCR product will be used for cloning using Thermo Scientific™ CloneJET™ PCR Cloning Kit (#K1231), the final extension step can be omitted.

TROUBLESHOOTING

For troubleshooting, visit **www.thermofisher.com.**

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable conversion of supercoiled plasmid DNA to a nicked form was observed.

Residual Activity Assay

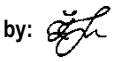
No detectable extension of labeled double stranded oligonucleotide with 5'-overhangs after incubation in the presence of dNTPs.

E. coli DNA Assay

No detectable *E.coli* DNA was observed.

Functional Assay

Performance in PCR is tested by the amplification of a 594 bp and 7.5 kb fragments of human genomic DNA.

Quality authorized by:  Jurgita Zilinskiene

By Royal Charter

Certificate of Registration

QUALITY MANAGEMENT SYSTEM - ISO 13485:2003 & EN ISO 13485:2012

This is to certify that:

Thermo Fisher Scientific
Units 4 to 8 Suffolk Drive
Fairwood Industrial Park
Ashford
Kent
TN23 4FD
United Kingdom

Holds Certificate Number: MD 635020

and operates a Quality Management System which complies with the requirements of ISO 13485:2003 & EN ISO 13485:2012 for the following scope:

The design, manufacture and supply of plastic consumables for in vitro diagnostic application.
The design, manufacture and repair of associated injection moulding tools.

For and on behalf of BSI:

Frank Lee, EMEA Compliance & Risk Director

Original Registration Date: 16/11/2015
Latest Revision Date: 10/06/2016

Effective Date: 10/06/2016
Expiry Date: 28/02/2019

...making excellence a habit.™

UKAS MANAGEMENT SYSTEMS 003

CERTIFICATE OF REGISTRATION

This is to certify that

Oxoid Company

1926 Merivale Rd., Unit 100, Nepean, Ontario K2G 1E8 Canada

Refer to Attachment to Certificate of Registration dated October 14, 2014 for additional certified sites

operates a

Quality Management System

which complies with the requirements of

ISO 13485:2003

for the following scope of registration

Design/Development, Manufacture and Distribution of the following in-vitro Diagnostic Medical Devices used in the isolation, identification and susceptibility testing of Micro-Organisms; - Ready-prepared, pre-poured microbiological culture media.

Certificate No.:	CERT-0074984	Original Date:	October 30, 2003
File No.:	025252	Effective Date:	October 13, 2014
		Expiry Date:	October 12, 2017

Chris Jouppi
President,
QMI-SAI Canada Limited

Samer Chaouk
Head of Policy, Risk and Certification

ISO 13485:2003

CMDCAS Recognized Registrar

ATTACHMENT TO
CERTIFICATE OF REGISTRATION

These sites are registered under Certificate No: CERT-0074984 issued on October 14, 2014

File No.

025252 **Oxoid Company**
1926 Merivale Rd., Unit 100, Nepean, Ontario K2G 1E8 Canada

Processes Covered at This Location: Design, development, manufacturing, sales, purchasing QA, and management processes.

1661902 **Oxoid Company**
1886 Merivale Road, Nepean, Ontario K2G 1E8 Canada

Dependant Processes Covered at This Location: Warehouse (Storage, Packaging & Shipping / Distribution.)

These registrations are dependent on Oxoid Company (File No. 025252) maintaining their scope of registration to ISO 13485:2003

SAI GLOBAL
INFORM. INSPIRE. IMPROVE.

TaqMan QSY probes

New quencher available for your qPCR probes

Applied Biosystems™ TaqMan™ QSY™ probes incorporate a proprietary nonfluorescent 3´ QSY quencher to provide maximal PCR performance in a multiplex format (Figure 1). Experience the sensitivity and specificity you know and expect from TaqMan™ Assays, with another great option for your real-time PCR assay designs.

Figure 1. QSY probe. The newly developed QSY quencher can be used in multiplex qPCR with FAM™, VIC™, ABY™, and JUN™ reporter dyes. The QSY quencher is nonfluorescent, leading to less background and improved quenching efficiency.

QSY probes are comparable to BHQ probes

Your current Black Hole Quencher™ (BHQ™) probe designs can easily be converted to QSY probes. Identical sequence designs can be used with similar performance using FAM dye (Figure 2) and improved performance using our ABY™ dye (Figure 3).

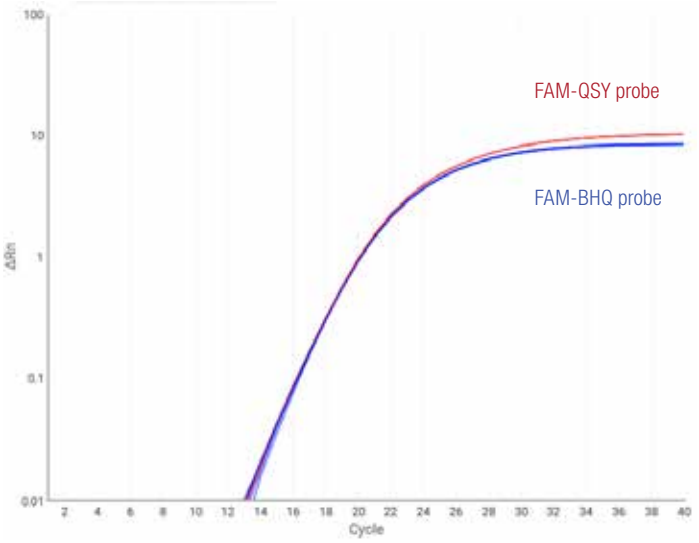


Figure 2. QSY probes have performance similar to that of BHQ probes. A FAM-QSY probe and a FAM-BHQ probe with identical oligonucleotide sequences and master mixes have similar C_t values.

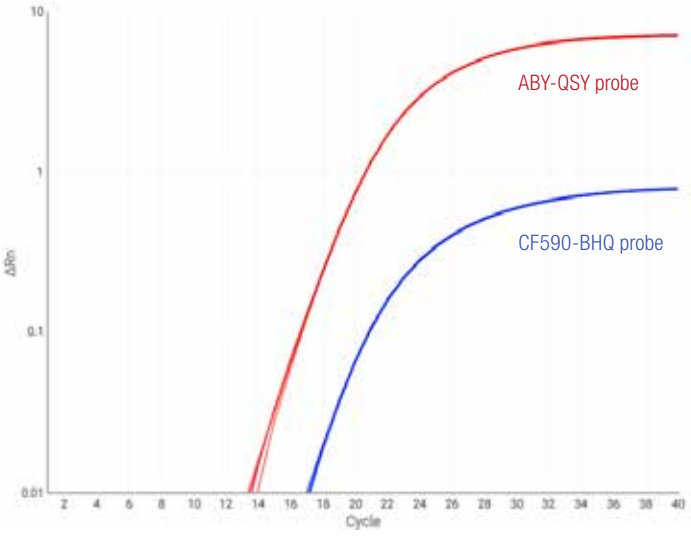


Figure 3. Improved performance in multiplex qPCR. In this multiplex experiment, the ABY-QSY probe shows a significantly lower C_t than the CF590-BHQ probe with an identical oligonucleotide sequence and master mix.

ThermoFisher SCIENTIFIC

applied biosystems

Four dye options optimized with our instruments for better sensitivity

TaqMan QSY probes can be ordered with FAM, VIC, and our proprietary ABY and JUN dyes, allowing amplification of up to 4 targets in a single reaction. All 4 dyes are optimized for the filter sets on Applied Biosystems™ real-time PCR instruments (Figure 4) and work together with minimal spectral overlap for optimal performance.

Performance without compromise

Multiplexing with TaqMan QSY probes enables cost savings and preservation of limited samples, and also yields comparable results between reactions performed in individual tubes and in 4-plex reactions, for a gene quantification experiment (Figure 5).

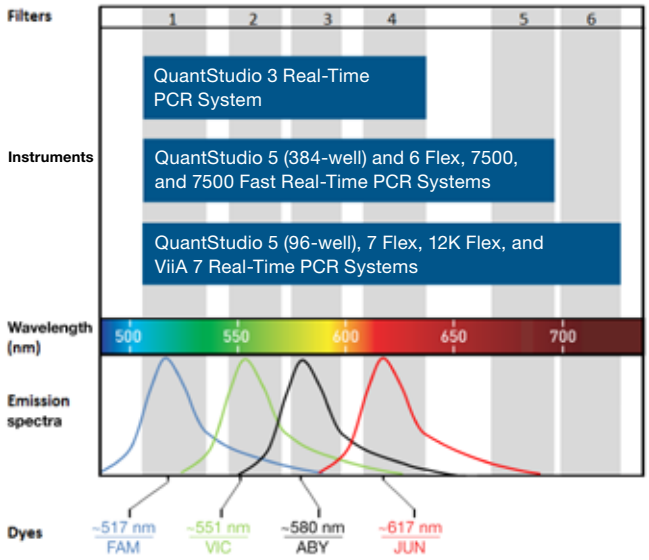


Figure 4. Fluorescence emission wavelengths used for multiplex real-time PCR. Emission spectra for FAM, VIC, ABY, and JUN dyes are shown in relation to regions of the spectrum detected by six filters available on Applied Biosystems real-time PCR instruments.

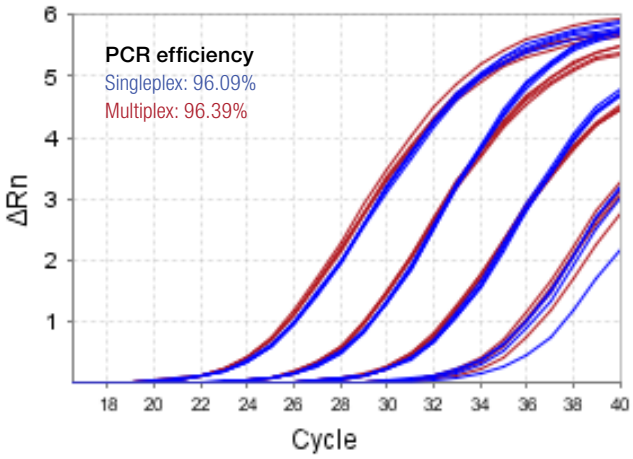


Figure 5. Comparable results for singleplex and multiplex assays. The amplification plot shows linear portions of the curves for 4 EGFR assays amplified in singleplex (blue) and 4-plex reactions (red) in a dilution series from 20,000 pg to 2 pg of reference colon cDNA per 10 µL reaction. PCR efficiencies are 96.09% for EGFR singleplex and 96.39% for EGFR 4-plex reactions.

Ordering information

Product	Quantity	Cat. No.
TaqMan QSY Probe	6,000 pmol	4482777
TaqMan QSY Probe	20,000 pmol	4482778
TaqMan QSY Probe	50,000 pmol	4482779

Product	Quantity	Cat. No.
TaqMan Multiplex Master Mix (2X)	5 mL	4461882
TaqPath 1-Step Multiplex Master Mix (4X)	5 x 1 mL	A28526
TaqPath 1-Step Multiplex Master Mix, No ROX (4X)	5 x 1 mL	A28522
Spectral Calibration Plate for Multiplex qPCR	1 plate	Various

Find out more at **thermofisher.com/multiplexqpcr**

ThermoFisher SCIENTIFIC