# 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*

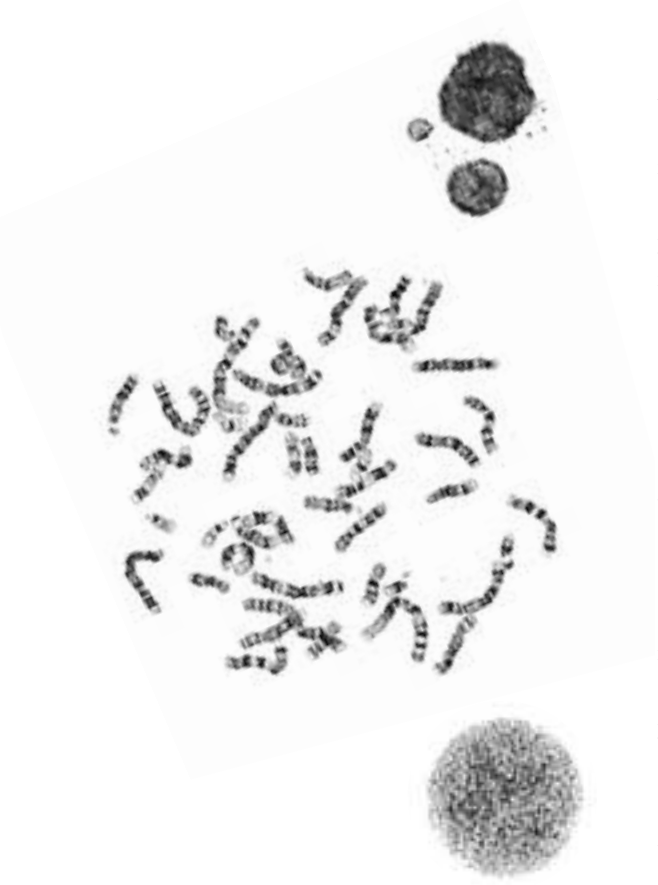

**Figure 1. Chromosome spread from normal bone marrow cells.** Cells were cultured in MarrowMAX™ Medium for 24 h.

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

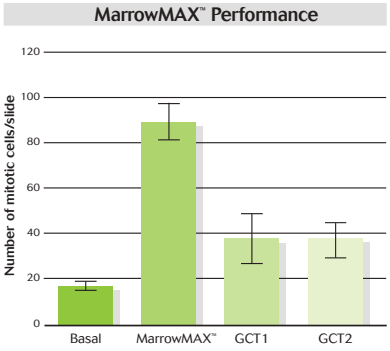


**Figure 2. Comparison of media for stimulation of mitotic cells.** Cells were cultured in basal medium without conditioned medium (Basal), MarrowMAX™ Medium which contains stromal cell-conditioned medium (MarrowMAX™), Supplier 1 Medium containing GCT-conditioned medium (GCT 1), and Supplier 2 Medium 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.

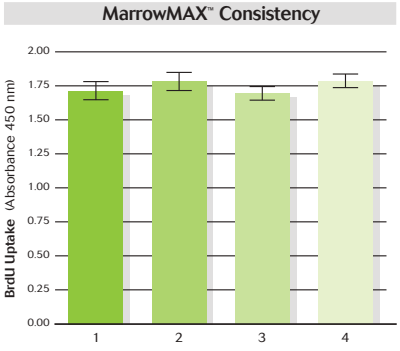


**Figure 3. MarrowMAX™ Medium consistency.** Normal bone marrow mononuclear cells were seeded at 1 × $10^5$ cells/ml in 4 different lots of MarrowMAX™ Medium. Results are mean ± SEM for N = 10.

## 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<br>17001-074 | 90 ml<br>450 ml |
| **AmnioMAX™-C100 Supplement,** liquid (supplement contains gentamicin) | 12556-015<br>12556-023 | 15 ml<br>75 ml |
| **PB-MAX™ Karyotyping Medium** (supplement contains gentamicin) | 12557-013<br>12557-021 | 100 ml<br>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

# GIBCO® media and culture supplements for cytogenetic analysis

- → Optimized and prequalified for cytogenetics
- → Provide high mitotic index
- → Deliver excellent chromosomal morphology
- → Produce clear, reproducible results that are easy to analyze and interpret

Every day, you make critical decisions based on what you see through a microscope. When your cytogenetics analysis is supported by GIBCO® media and culture supplements, you can be confident in the conclusions you reach.

You'll get clear, reproducible results that are simple to analyze and interpret when you use the most trusted cell culture media and reagents for cytogenetics: MarrowMAX™, AminoMAX™, and PB-MAX™ products.

## Superior performance

- → High mitotic index and superior chromosomal morphology (Figure 1)
- → Outperforms commercially available giant cell tumor conditioned medium (GCT-CM) (Figure 2)
- → Consistent lot-to-lot performance (Figure 3)

## Convenience

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

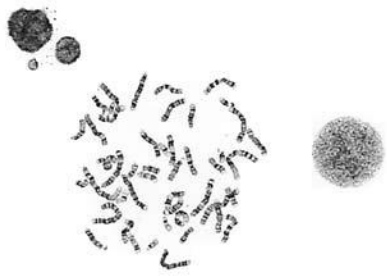

Figure 1—Chromosome spread from bone marrow cells. Cells were cultured in MarrowMAX™ Medium for 24 hours, and G-banding analysis was performed.

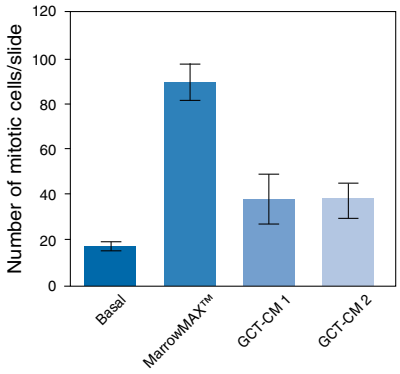


Figure 2—Performance of MarrowMAX™ Medium. Cells were cultured in: basal medium without conditioned medium; MarrowMAX™ Medium; Supplier 1 Medium (GCT-CM 1); and Supplier 2 medium (GCT-CM 2) (both Supplier 1 and Supplier 2 media contain GCT-conditioned medium). Mitotic cells were assayed 24 hours after plating.

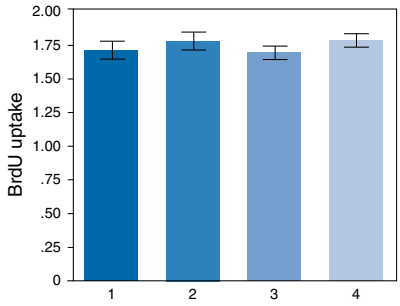


Figure 3—Consistency of MarrowMAX™ Medium. Normal bone marrow mononuclear cells were seeded at 1 x $10^5$ cell/ml in 4 different lots of MarrowMAX™ Medium. BrdU uptake was measured by absorbance at 405 nm. Results are mean ± SEM for n = 10.

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# Cytogenetic and Diagnostic Testing

## Peace of mind with each product

- → 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 to deliver clear, reproducible results in standard clinical cytogenetic protocols
- → Extended shelf life of 18 months when stored unopened at –20°C and 60 days stored at 4°C

## We know what matters

At Invitrogen, we understand the high level of service and support required in cytogenetics laboratories, so we strive to help you in every way possible. Have questions? Need data? Contact Invitrogen's cytogenetics specialists at 1 800 955 6288 or visit www.invitrogen.com/cytogenetics.

## Ordering Information

| Product | Size | Cat. no. |
|---|---|---|
| MarrowMAX™ Bone Marrow Medium * (contains gentamicin) | 100 ml | 12260-014 |
| AmmioMAX™–II Complete Medium (contains gentamicin) | 100 ml | 11269-016 |
| AmnioMAX™–C100 Complete Medium (system)<br>The system contains both the basal medium (90 ml) and the supplement (15 ml) (supplement contains gentamicin) | 1 set | 12558-011 |
| AmnioMAX™–C100 Basal Medium, liquid | 90 ml<br>450 ml | 17001-082<br>17001-074 |
| AmnioMAX™–C100 Supplement, liquid (contains gentamicin) | 15 ml<br>75 ml | 12556-015<br>12556-023 |
| PB-MAX™ Karyotyping Medium (contains gentamicin) | 100 ml<br>500 ml | 12557-013<br>12557-021 |
| KaryoMAX™ Colcemid® Solution, liquid (10 mg/ml), in HBSS | 10 ml | 15210-040 |
| KaryoMAX™ Colcemid® Solution, liquid (10 mg/ml), in PBS | 10 ml | 15212-012 |
| KaryoMAX™ Giemsa Stain Stock Solution | 100 ml | 10092-013 |
| Fungizone® Antimycotic, liquid | 20 ml | 15290-018 |
| Phytohemagglutinin (M Form) (PHA), lyophilized † | 10 ml | 10576-015 |

* This product is subject to Limited Use Label License No. 31. These products are for in vitro use and are not intended for human or animal therapeutic use. Uses other than the labeled intended use may be a violation of federal law. † The noted products are for laboratory use only and not for diagnostic use. The safety and efficacy of these products in diagnosis or other clinical uses has not been established. Colcemid® is a registered trademark of CIBA-GEIGY Corporation.

Visit us at www.invitrogen.com/cytogenetics to learn about related reagents for cytogenetic cell culture.

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GIBCO®
invitrogen cell culture

www.invitrogen.com

# Certificate of Analysis

**QC Code: GIBCO**

KaryoMAX™ Giemsa Stain

| | |
|---|---|
| **Lot Number:** | 2448626 |
| **Item Number:** | 10092 |
| **Expiration Date:** | 2025-07 |
| **Storage Temp:** | 15 to 30C |
| **Storage Instructions:** | Protect from light |
| **Originated From:** | K230611 |

For Research Use or Further Manufacturing. Not for diagnostic use or direct administration into humans or animals.

| TEST | TEST ID | SPECIFICATION | RESULT | UNITS |
|---|---|---|---|---|
| **Chrom 10 Banding Resolution Act Std. Dev** | PERBLD0007 | Check and Record | 4.017 | |
| **Chrom 10 Banding Resolution Actual** | PERBLD0006 | Check and Record | 40.100 | |
| **Chrom 10 Banding Resolution Cont Std Dev** | PERBLD0009 | Check and Record | 3.201 | |
| **Chrom 10 Banding Resolution Control** | PERBLD0008 | Check and Record | 40.200 | |
| **Peripheral Blood Performance Testing** | PERBLD0001 | Acceptable | Acceptable | |
| *- Testing is performed on the raw material.* | | | | |

Read SDS.

Storage and handling precautions: Limit product exposure to air. Cap tightly.

Recommended Dilution Instructions: Peripheral Blood Chromosomes: 3 mL Giemsa Stain, 48.5 mL Gurr Buffer (pH 6.8)

NOTICE: Effective 22MAY2023 the product description has been updated to KaryoMAX(TM) Giemsa Stain per ECR1101109. If you have questions regarding this change, please contact Thermo Fisher Scientific Technical Support at 1-800-955-6288 in North America or Techsupport@thermofisher.com globally.

NOTICE: Effective 13MAY2022 the Intended Use Statement has been updated to For Research Use or Further Manufacturing. Not for use or direct administration into humans or animals per ECR1087081. If you have questions regarding this change, please contact Thermo Fisher Scientific Technical Support at 1-800-955-6288 in North America or Techsupport@thermofisher.com globally.

Quality Systems Department Date: 13-Dec-2023

## References


- PERBLD0007: The chormosome 10 banding resolution represents the standard deviation for the mean value for the actual lot. This is a parameter assessed by the Peripheral Blood Performance Test, see reference.
- PERBLD0006: The chormosome 10 banding resolution represents the mean value for the actual lot. This is a parameter assessed by the Peripheral Blood Performance Test, see reference.
- PERBLD0009: The chromosome 10 banding resolution represents the standard deviation for the mean value for the control lot. This is a parameter assessed by the Peripheral Blood Performance Test, see reference.
- PERBLD0008: The chromosome 10 banding resolution represents the mean value for the control lot. This is a parameter assessed by the Peripheral Blood Performance Test, see reference.

- PERBLD0001: The Karyomax range of media and reagents are evaluated for performance using a test system comparable to the protocols routinely used in cytogenetic laboratories. Test samples of the reagent or media are run in parallel against a previously qualified control lot in a system pertinent to the normal usage in a cytogenetic laboratory. The resulting preparations are analyzed for statistical defference in mitotic index and colony quantity and quality or banding resolution where relevant.

**Thermo Fisher Scientific Baltics UAB**
V. A. Graiciuno 8, Vilnius, LT-02241 Lithuania
Phone +370 700 55131, Fax +370 700 55132
www.thermofisher.com
cofarequests@thermofisher.com
Company code 122351387, VAT code LT223513811

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# CERTIFICATE OF ANALYSIS

## EP1702 DreamTaq Hot Start DNA Polymerase, 500 U

**Packaging Lot:** **2902495**
Expiry Date: 31.10.2025 (DD.MM.YYYY)
Storage: at -20±5°C

**Filling lots for components in package:**

| Lot | Quantity | Description |
|---|---|---|
| 2863898 | 0.5 kU | DreamTaq HS DNA Polymerase |
| 2877165 | 2 × 1.25 mL | 10X DreamTaq Buffer |

## QUALITY CONTROL

| Parameter | Method | Requirement | Result |
|---|---|---|---|
| Unit concentration | One unit of enzyme incorporates 10 nmoles of dNTPs into a polynucleotide fraction at 74 ºC in 30 minutes. | 5.5 ± 0.5 U/µl | Conforms |
| Endodeoxyribonuclease Assay | No detectable conversion of supercoiled plasmid DNA to a nicked form was observed. | Not detectable | Conforms |
| Residual Activity Assay | No detectable extension of labeled double stranded oligonucleotide with 5'-overhangs after incubation in the presence of dNTPs. | Not detectable | Conforms |
| E. coli DNA | No detectable E.coli DNA was observed. | Not detectable | Conforms |
| Functional Assay | Performance in PCR is tested by the amplification of a 594 bp and 7.5 kb fragments of human genomic DNA. | Reactions produce specific PCR products | Conforms |

ISO CERTIFICATION
Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

**Quality authorized by QC:** J. Žilinskienė

# DreamTaq DNA polymerases

Thermo Scientific™ DreamTaq™ DNA polymerases are available in standard and hot-start varieties and multiple formats for everyday PCR. Choose DreamTaq Hot Start DNA Polymerase for higher specificity, sensitivity, and yield compared to the standard DreamTaq DNA Polymerase. DreamTaq Hot Start DNA Polymerase also allows for room-temperature reaction setup and reaction stability.

## General recommendations for PCR setup

### Template DNA

- The optimal amount of template DNA for a 50 µL reaction is 0.01–1.0 ng for both plasmid and phage DNA, and 0.1–1.0 µg for genomic DNA.

### Primers

- The recommended concentration range for the PCR primers is 0.1–1.0 µM.
- For degenerate primers and primers used for long-range PCR, use higher primer concentrations in the range of 0.3–1.0 µM.

### $MgCl_2$ concentration

- 10X DreamTaq Buffer and DreamTaq™ PCR Master Mixes include $MgCl_2$ for a final reaction concentration of 2.0 mM, which is optimal for the majority of PCR reactions.
- If the DNA samples contain EDTA or other metal chelators, the $Mg^{2+}$ concentration should be increased accordingly (one molecule of EDTA binds one $Mg^{2+}$ ion).

### Did you choose the right format for your workflow?

| Format | DreamTaq DNA Polymerase | DreamTaq Hot Start DNA Polymerase |
|---|---|---|
| Stand-alone enzyme—flexibility in PCR setup | Colorless (Cat. No. EP0701/EP0702)* | Colorless (Cat. No. EP1701/EP1702)* |
| | Green** (Cat. No. EP0711/EP0712)* | Green** (Cat. No. EP1711/EP1712)* |
| DreamTaq PCR Master Mix—convenience with ready-to-use mix | Colorless (Cat. No. K1071/K1072) | Colorless (Cat. No. K9011/K9012) |
| | Green** (Cat. No. K1081/K1082) | Green** (Cat. No. K9021/K9022) |

* Additional product sizes available.

** Green gel loading dye already added.

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## Reaction setup

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Place a thin-walled PCR tube on ice for a reaction using standard DreamTaq DNA Polymerase, or mix and add the components, as shown in the table, for each 50 µL reaction. Reaction setup on ice is not required for DreamTaq Hot Start DNA Polymerase or master mix.
3. Gently vortex the samples and spin down.
4. When using a thermal cycler that does not have a heated lid, overlay the reaction with 25 µL of mineral oil.
5. Perform PCR using the recommended thermal cycling conditions outlined below.

| Components | DreamTaq/ DreamTaq Hot Start DNA Polymerase | DreamTaq/ DreamTaq Hot Start PCR Master Mix (2X) |
|---|---|---|
| 10X DreamTaq Buffer* | 5 µL | — |
| dNTP Mix, 2 mM (Cat. No. R0241) | 5 µL (0.2 mM of each) | — |
| Forward primer | 0.1–1.0 µM | 0.1–1.0 µM |
| Reverse primer | 0.1–1.0 µM | 0.1–1.0 µM |
| Template DNA | 10 pg–1 µg | 10 pg–1 µg |
| DreamTaq DNA Polymerase/DreamTaq PCR Master Mix (2X) | 1.25 U | 25 µL |
| Water, nuclease-free | To 50 µL total | To 50 µL total |
| Total volume | 50 µL | 50 µL |

* 10X DreamTaq Buffer contains 20 mM $MgCl_2$, which is optimal for most applications. A final $MgCl_2$ concentration of 2.0 mM is generally ideal for PCR. If additional optimization is required, the $MgCl_2$ concentration can be further increased up to 4.0 mM with addition of 25 mM $MgCl_2$ (Cat. No. R0971). The volume of water should be reduced accordingly.

## Tips for optimizing cycling conditions

- For GC-rich DNA templates, the denaturation step can be prolonged to 3–4 min. DNA denaturation can also be enhanced by the addition of 5–10% glycerol, 5% DMSO, 1% formamide, or 1.0–1.5 M betaine.
- For amplification of templates >6 kb, we recommend reducing the extension temperature to 68°C.
- If the PCR product will be cloned into a TA vector such as in the Thermo Scientific™ InsTAclone™ PCR Cloning Kit (Cat. No. K1213), the final extension step may be prolonged to 15 min to enable the complete 3´ dA tailing of the PCR product.

## Cycling conditions

| Step | DreamTaq DNA Polymerase/ Master Mix | | DreamTaq Hot Start DNA Polymerase/ Master Mix | | Number of cycles |
|---|---|---|---|---|---|
| | Temp. | Time | Temp. | Time | |
| Initial denaturation | 95°C | 1–3 min | 95°C | 1–3 min | 1 |
| Denaturation | 95°C | 30 sec | 95°C | 30 sec | 25–40 |
| Annealing | $T_m$ – 5°C | 30 sec | $T_m$* | 30 sec | |
| Extension** | 72°C | 1 min | 72°C | 1 min | |
| Final extension | 72°C | 5–15 min | 72°C | 5–15 min | 1 |

* Depends on the primer $T_m$ values. Use the $T_m$ calculator at **thermofisher.com/tmcalculator**

** 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.

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

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# DreamTaq Hot Start DNA Polymerase

## The hot-start polymerase for everyday research

New Thermo Scientific™ DreamTaq™ Hot Start DNA Polymerase offers a great balance between performance and value. Designed for consistently robust and reliable amplification, DreamTaq Hot Start DNA Polymerase can help you more easily get the results you're looking for, with virtually any template, application, or target.

### Why use hot-start PCR?

- Prevents amplification of nonspecific products
- Amplifies low-abundance targets
- Provides convenient room-temperature setup

### Why use DreamTaq Hot Start DNA Polymerase?

DreamTaq Hot Start DNA Polymerase is the hot-start version of our enhanced Thermo Scientific™ DreamTaq™ DNA Polymerase. Like our standard DreamTaq DNA Polymerase, this hot-start polymerase offers higher yields and longer amplicons than conventional *Taq*-based products. In addition, due to the hot-start modification, DreamTaq Hot Start DNA Polymerase has been engineered to provide increased sensitivity and specificity.

### Features:

- Minimized optimization of primer annealing temperatures
- Optimized DreamTaq™ buffer, which includes 20 mM $MgCl_2$
- Ability to use same cycling conditions as used with conventional *Taq* polymerase
- Wide range of amplicon lengths
- 2X master mix formats
- Direct loading options
- Compatibility with most PCR applications

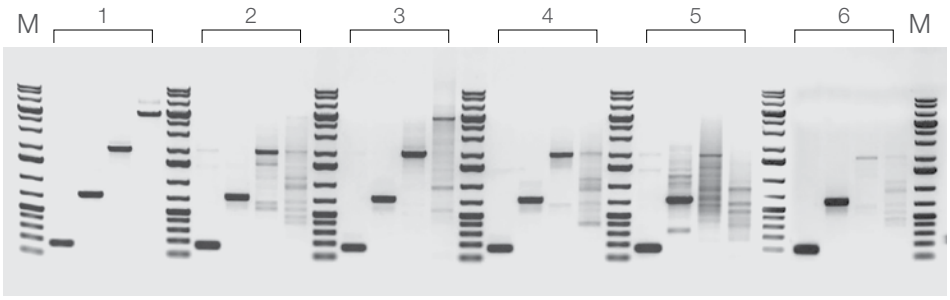


**Figure 1. Robust amplification of human genomic DNA.** DreamTaq Hot Start DNA Polymerase produces more product, cleaner bands, and longer amplicons than hot-start DNA polymerases from other suppliers. Amplification products (160 bp, 727 bp, 2 kb, or 5 kb) from human genomic DNA are shown in the figure above.
M: GeneRuler 1 kb Plus DNA Ladder. 1. DreamTaq Hot Start DNA Polymerase; 2. Promega GoTaq G2 Hot Start Polymerase; 3. NEB One*Taq* Hot Start DNA Polymerase; 4. TaKaRa *Taq* DNA Polymerase Hot Start Version; 5. Kapa Biosystems KAPA2G Robust HotStart PCR Kit; 6. Bioline MyTaq HS DNA Polymerase.

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## Technical details

- Amplifies from as little as 3 pg human genomic DNA
- Routinely amplifies up to 6 kb genomic DNA and 20 kb lambda DNA
- Generates 3´-dA overhangs
- Incorporates dUTP and modified nucleotides

## Usage and applications

Choose DreamTaq Hot Start DNA Polymerase for the amplification of DNA from plasmid, viral, or complex genomic templates. Common applications include:

- Colony PCR
- Genotyping
- RT-PCR
- Generation of PCR products for TA cloning

## Why use green?

The Thermo Scientific™ DreamTaq™ Green Buffer (10X) supports direct gel loading of PCR products. The two tracking dyes and a density reagent in the green buffer do not interfere with PCR performance and are compatible with downstream applications including DNA sequencing, ligation, and restriction digestion.

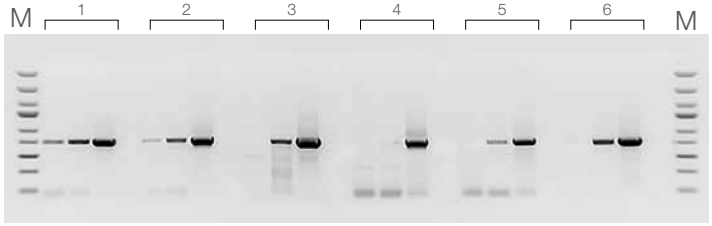


**Figure 2. High sensitivity.** DreamTaq Hot Start DNA Polymerase amplifies from lower template amounts than hot-start DNA polymerases from other suppliers. Each set of PCR reactions contained either 3 pg, 30 pg, or 3 ng of human genomic DNA.
M: GeneRuler Express DNA Ladder. 1. DreamTaq Hot Start DNA Polymerase; 2. TaKaRa *Taq* DNA Polymerase Hot Start Version; 3. Kapa Biosystems KAPA2G Robust HotStart PCR Kit; 4. Bioline MyTaq HS DNA Polymerase; 5. NEB One*Taq* Hot Start DNA Polymerase; 6. Promega GoTaq G2 Hot Start Polymerase.

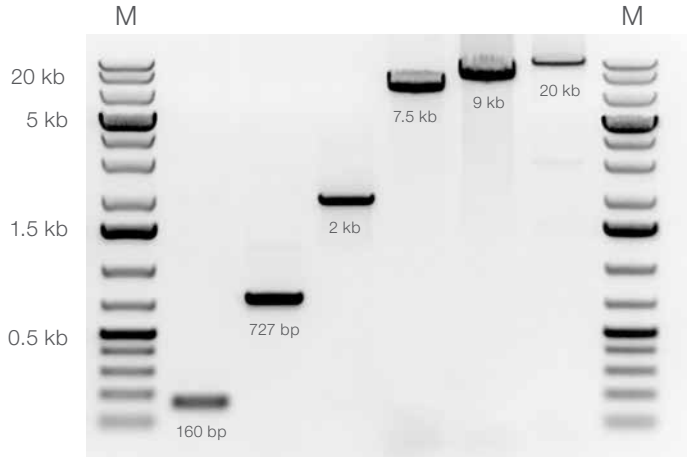


**Figure 3. Consistent and reliable amplification.** DreamTaq Hot Start DNA Polymerase amplifies human genomic DNA with high specificity up to 9 kb amplicons. Even longer 20 kb amplicons can be amplified with lambda DNA templates.
M: Thermo Scientific™ GeneRuler™ 1 kb Plus DNA Ladder.

## Ordering information

| Product | Quantity | Cat. No. |
|---|---|---|
| DreamTaq Hot Start DNA Polymerase | 200 U | EP1701 |
| | 500 U | EP1702 |
| | 2,500 U | EP1703 |
| | 4 x 2,500 U | EP1704 |
| DreamTaq Hot Start PCR Master Mix | 200 reactions | K9011 |
| | 1,000 reactions | K9012 |

| Product | Quantity | Cat. No. |
|---|---|---|
| DreamTaq Hot Start Green DNA Polymerase | 200 U | EP1711 |
| | 500 U | EP1712 |
| | 2,500 U | EP1713 |
| | 4 x 2,500 U | EP1714 |
| DreamTaq Hot Start Green PCR Master Mix | 200 reactions | K9021 |
| | 1,000 reactions | K9022 |

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

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# TaqMan® multiplex qPCR

## Accurate, sensitive, and as efficient as traditional qPCR

In this application note, we show:

- A new multiplexing solution with probes having ABY® and JUN® reporter dyes and a QSY® nonfluorescent quencher
- An optimized TaqMan® Multiplex Master Mix that can be used for genotyping and gene expression analysis
- The new multiplexing solution is as accurate and sensitive as singleplex PCR on the same loci
- Multiplex qPCR assay results comparable to singleplex or duplex qPCR results can be obtained with less starting input material per assay

### Introduction

Current analyses of cell and tissue functionality require extracting as much information as possible from materials that are often limited. For example, samples such as tumor biopsies are difficult to collect and usually yield only a small amount of usable nucleic acid. Singleplex qPCR has been the gold standard for analyzing clinical research samples on the nucleic acid level, and has been invaluable in extending the limits of biological knowledge for more than a quarter century.

| Genotyping | Gene expression |
|---|---|
| • Obtain genomic DNA from:<br>– SW620 colorectal adenocarcinoma cell line<br>– Panc 03.27 pancreatic adenocarcinoma cell line<br>– MDA-MB-468 breast adenocarcinoma cell line<br>– 23 normal Coriell cell lines for reference<br>• Design multiplex genotyping assays:<br>– *TP53* R273H<br>– *KRAS* G12V<br>• Compare results from multiplex to singleplex SNP assays run on QuantStudio® 7 system | • Obtain total RNA from:<br>– SW620 cells<br>– Human total colon RNA<br>• Convert to cDNA using SuperScript® VILO™ cDNA Synthesis Kit<br>• Design multiplex gene expression assays (gene selection was done following profiling of the samples using TaqMan® OpenArray® Human Signal Transduction Panel run on QuantStudio® 12K Flex Real-Time PCR System)<br>• Compare results from multiplex to singleplex gene expression assays run on QuantStudio® 7 system |

**Figure 1. Experimental approach for two different multiplexing applications.**

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However, the limited amount of nucleic acid obtained from clinical research specimens often forces choices to be made about how best to utilize these precious samples. Furthermore, if the sample is limited, the number of loci that can be analyzed is also limited, reducing the amount of information that can be extracted from the sample. Finally, the additional time and materials required to set up multiple single-assay reactions could increase the expense of a complex project significantly.

Multiplex qPCR analysis of nucleic acids, a strategy where more than one target is amplified and quantified from a single sample aliquot, is an attractive solution to these problems. In multiplex PCR, a sample aliquot is queried with multiple probes that contain fluorescent dyes in a single PCR reaction. This increases the amount of information that can be extracted from that sample. With multiplex qPCR, significant savings in sample and materials can be realized.

In this application note, we describe a multiplex qPCR solution for SNP genotyping and mRNA gene expression analysis. To demonstrate the versatility of the new multiplex solution we characterized oncogenic lesions in the colorectal adenocarcinoma cell line SW620, the pancreatic adenocarcinoma cell line Panc 03.27, and the breast adenocarcinoma cell line MDA-MB-468. These cells are highly tumorigenic when transplanted in nude mice, are known to harbor mutations in the *KRAS* and *TP53* genes, and thus are good models for tumor dynamics [1,2]. First, we verified that the cell lines have pathogenic alleles of the *KRAS* and *TP53* loci, but that 23 normal DNA samples (Coriell) do not. Next, we simultaneously profiled the expression levels of three sets of four different genes in different multiplex groups in SW620 cells and compared them to normal colon total RNA as a reference sample. The overall experimental approach is shown in Figure 1.

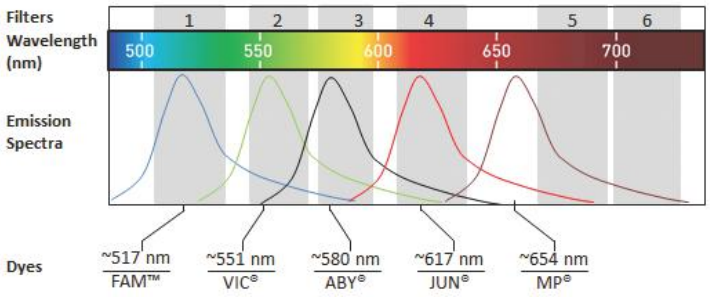



**Figure 2. Fluorescence emission spectra of different dyes used for multiplex qPCR.**

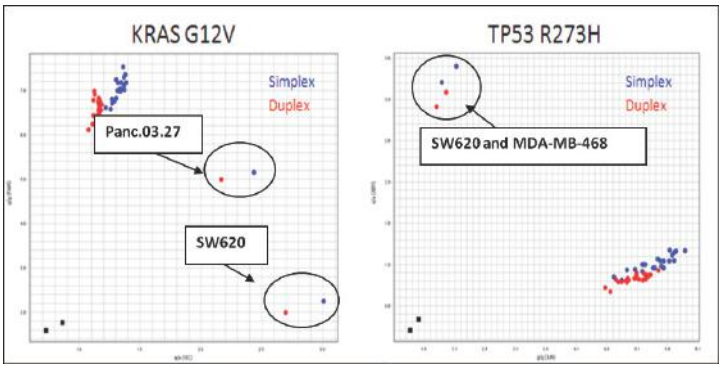



**Figure 3. Comparing *KRAS* G12V and *TP53* R273H genotyping in singleplex (blue) and duplex (red) reactions across three cancer cell lines and 23 normal samples. (A)** Panc 03.27 and SW620 are shown to be heterozygous and homozygous for *KRAS* G12V mutation (G>T), respectively. **(B)** MDA-MB-468 and SW620 are homozygous for a G>A mutation in *TP53*.

Multiplex qPCR requires introduction of new dyes. We have introduced a new multiplexing solution with four reporter dyes, a new quencher, and a new master mix. The ABY® and JUN® reporter dyes complement our existing offering of FAM™ and VIC® reporter dyes for multiplexing. These four reporter dyes with distinct spectra are optimized to work together with minimal spectral overlap (Figure 2). We have developed the new QSY® nonfluorescent quencher in order to facilitate multiplex qPCR with the four dyes. Finally, we have developed a new TaqMan® Multiplex Master Mix to be compatible with these four reporter dyes and that gives accurate and precise results. The master mix uses MUSTANG PURPLE® (MP) as a passive reference dye and AmpliTaq® UP polymerase, which only requires a 20-second activation time. This multiplexing solution is compatible with the QuantStudio® 6, 7, and 12K Flex systems, as well as the ViiA™ 7 and 7500/7500 Fast Real-Time PCR Systems.

## Application of multiplex qPCR to genotyping

To demonstrate the utility of multiplex PCR, we first examined the genotypes of different cell lines at two different loci. We used the predesigned TaqMan® SNP Genotyping Assay (C_31385346_10) that interrogates *KRAS* G12V with FAM™ dye– and VIC® dye–labeled NFQ-MGB probes and a custom-made SNP assay to *TP53* R273H that contains custom ABY®-QSY® and *JUN®-QSY®* probes. Each SNP assay was run individually and also run together in a multiplex reaction with the cell–line derived gDNAs. Reactions were performed on a QuantStudio® 7 instrument using indentical thermocycling profiles (95°C 20 s, [95°C 5 s + 60°C 30 s] x 40 cycles). The following parameters served as the criteria for comparison between individual assay and multiplex assay reactions: call concordance and signal intensity (ΔRN).

The resulting genotype calls are illustrated in Figure 3. The 100% call concordance between singleplex and duplex reactions shows that the new multiplex reagents provide similar efficiencies to the traditional singleplex reagents. Notably, the two single-assay reactions required a total of 20 ng of gDNA, whereas the multiplex assay reaction required only 10 ng. This illustrates how multiplex PCR is capable of extracting more information from a smaller amount of sample. Together, these results demonstrate that duplex assay genotyping using TaqMan® Multiplex Master Mix and the newly developed ABY® dye–labeled and JUN® dye–labeled QSY® probes is an accurate and attractive solution to analysis of limited or precious samples. In most cases, multiplex optimization is probably not necessary but refer to the TaqMan® Multiplex Master Mix User Guide for optimization recommendations if needed.

## Application of multiplex qPCR to gene expression analysis

We next evaluated the ability of multiplex qPCR to analyze mRNA levels. For these experiments, we took an approach similar to that used to identify genes that are differentially regulated in cancer. We obtained purified RNA from SW620 colon carcinoma cells and normal human colon. Both RNA samples were converted to cDNA using the SuperScript® VILO™ cDNA Synthesis Kit. To identify candidates that are differentially expressed in these samples, we performed an initial profiling experiment using the TaqMan® OpenArray® Human Signal Transduction Panel on the QuantStudio® 12K Flex Real-Time PCR System. This panel contains 573 unique assays related to signaling pathways and 24 reference assays. Genes that showed more than two-fold change in measured levels were selected for the multiplex gene expression study. Eight gene targets from the OpenArray® profiling results were chosen and gene expression assays were designed using a custom design process for QSY®, non-MGB probes. Assays for these targets plus *B2M* (used as the endogenous control) were synthesized; each had a different dye at the 5′ end and the QSY® quencher on the 3′ end (Table 1). Primer-probe mixes were formulated at 20x concentration for reaction concentrations of 300 nM forward primer, 300 nM reverse primer, and 250 nM probe.

**Table 1. Gene expression assays for the multiplex gene expression application.** Assay IDs were submitted to our custom services for redesign with QSY® probes.

| Gene symbol, assay ID | Reporter dye/quencher |
|---|---|
| *BAX, Hs00180269_m1* | JUN® |
| *EP3000, Hs00914223_m1* | VIC® |
| *EGFR, Hs01076078_m1* | FAM™ |
| *CFLAR, Hs01116280_m1* | JUN® |
| *MYC, Hs00905030_m1* | VIC® |
| *AKT2, Hs01086102_m1* | FAM™ and JUN® |
| *MALT1, Hs00198984_m1* | VIC® |
| *EGR1, Hs00152928_m1* | FAM™ |
| *B2M*, 4310886E | ABY® |

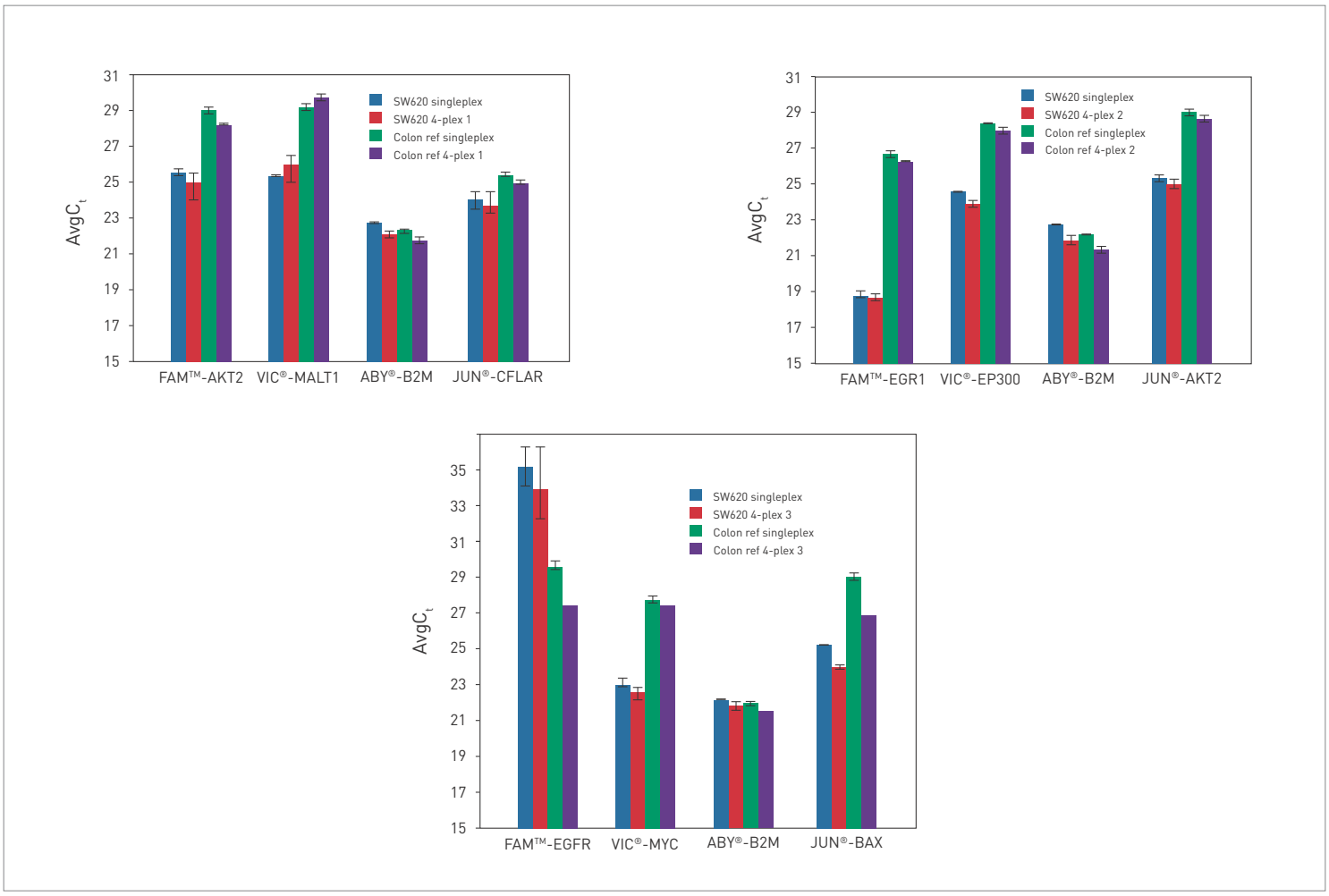



Figure 4. Comparison of average $C_t$ (n = 4) of nine loci and one reference gene from SW620 cell line RNA and normal colon RNA in singleplex and multiplex qPCR format.

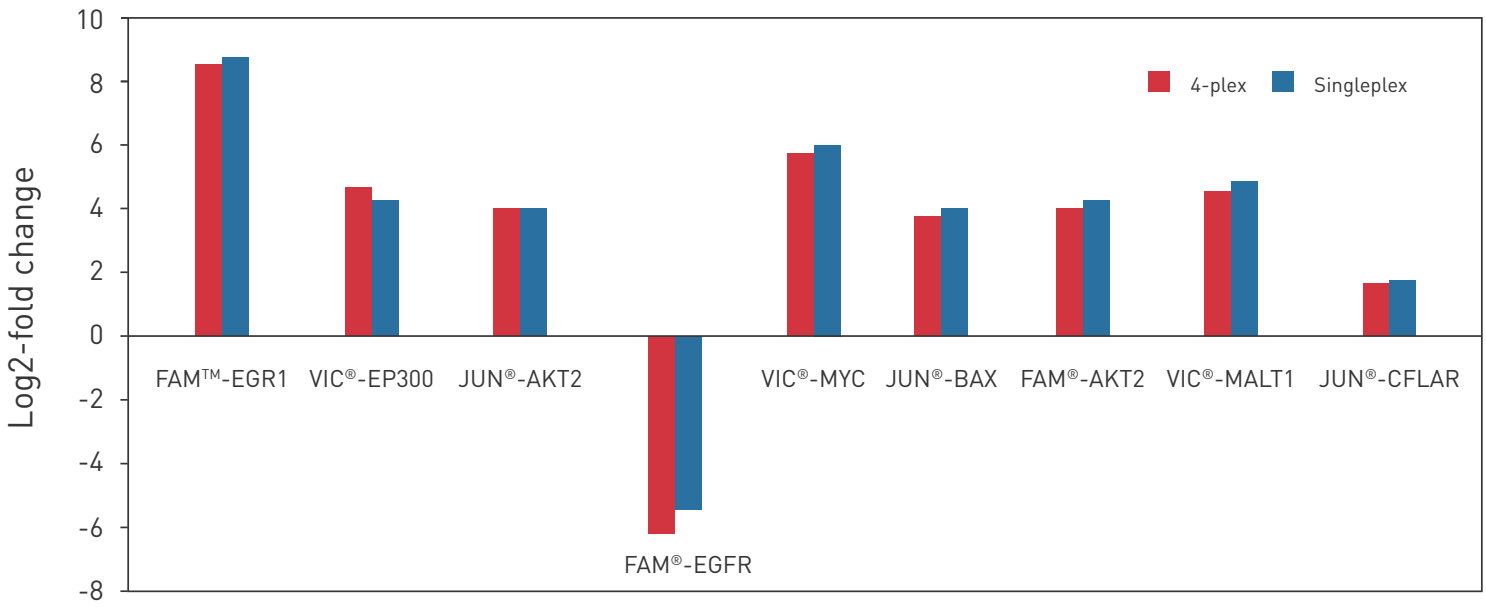
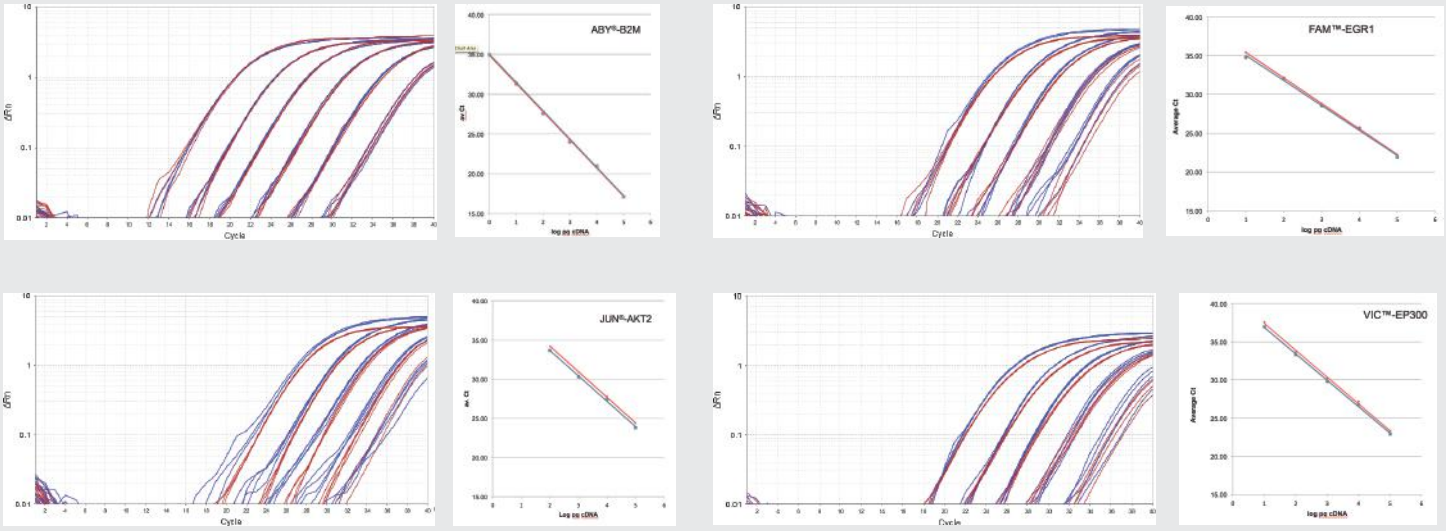



Figure 5. Fold change comparison of nine targets normalized to *B2M* in singleplex and multiplex qPCR format.

## Comparison of singleplex and multiplex gene expression experiment

It's important to validate that a multiplex gene expression experiment provides similar results to singleplex experiments before performing any sample comparisons. To do this, we performed serial dilutions of the colon reference cDNA. Reactions (n = 4) were run over four logs for each assay in singleplex and 4-plex format. The TaqMan® Multiplex PCR Optimization guide provides guidance on how to evaluate data such as $C_t$ values, PCR efficiency, coefficient of correlation, and standard deviation.




| | PCR efficiency | | Coefficient of correlation ($R^2$) | |
|---|---|---|---|---|
| | Singleplex | 4-plex | Singleplex | 4-plex |
| FAM™-EGR1 | 101.21% | 104.18% | 0.997 | 0.996 |
| VIC®-EP300 | 92.57% | 94.54% | 0.996 | 0.994 |
| ABY®-B2M | 91.04% | 91.54% | 0.999 | 0.999 |
| JUN®-AKT2 | 102.35% | 103.38% | 0.993 | 0.995 |

**Figure 6. EGR1 assay using FAM™ dye, EP300 assay using VIC® dye, B2M assay using ABY® dye, and AKT2 assay using JUN® assay were analyzed in singleplex and 4-plex reactions.** Amplification was performed using a serial dilution of reference colon cDNA from 20,000 pg to 2 pg per 10 µL reaction. In all four amplification plots, blue refers to 4-plex reactions and red represents singleplex reactions. The table summarizes the PCR efficiency and coefficient of correlation for each assay. Data for other multiplex experiments are not shown.

TaqMan® Multiplex Master Mix was used for amplification of both the single-target and multiple-target reactions. Following the TaqMan® Multiplex Master Mix User Guide, 15 ng of each cDNA was used in a 10 µL reaction in a 384-well plate. Thermal cycling parameters were 95°C (20 s) followed by 40 cycles of 95°C (5 s) and 60°C (40 s). Real-time PCR data was collected using a QuantStudio® 7 Flex system. Three 4-plex reactions were performed: 4-plex 1 contained *AKT2* (FAM™ dye), *MALT1* (VIC® dye), *B2M* (ABY® dye), and *CFLAT* (JUN® dye) assays; 4-plex 2 contained *EGR1* (FAM™ dye), *EP300* (VIC® dye), *B2M* (ABY® dye), and *AKT2* (JUN® dye) assays; 4-plex 3 contained *EGFR* (FAM™ dye), *MYC* (VIC® dye), *B2M* (ABY® dye), and *BAX* (JUN® dye). *AKT2* gene assay was tested with two different dyes: FAM™ dye (4-plex 1) and ABY® dye (4-plex 2).

We calculated the average $C_t$ values (n = 4) for each assay in single reactions and 4-plex reactions in both samples (Figure 4). The correlation between the singleplex and multiplex measurements is evident; there was little or no significant difference between the two different measurement strategies. Note that a total of 135 ng of cDNA was needed for the nine singleplex reactions, while only 45 ng was required for the three multiplex reactions.

Next, the average $C_t$ for each assay-sample combination was used to determine the $\Delta C_t$ between the target gene and beta-2 microglobulin (*B2M*) control gene to normalize for RNA input amount. Finally, to measure the fold change between the SW620 cells and normal colon cells, the $\Delta\Delta C_t$ was calculated from the $\Delta C_t$ of the sample minus the $\Delta C_t$ of the reference for each assay (Figure 5). One gene, *EGFR*, was down-regulated (71-fold) while seven genes were

up-regulated (*EGR1*: 362-fold, *EP300*: 21-fold, *AKT2*: 16-fold, *MYC*: 51-fold, *BAX*: 11-fold, *MALT1*: 21-fold, and *CFLAR*: 3-fold) and all were in concordance with singleplex results (see side bar, Figure 6). Interestingly, these eight genes are known to play roles in colon cancer [3–10].

Together, these results demonstrate that (1) multiplex PCR is as accurate at measuring differences in transcript levels *as* singleplex PCR, (2) sample *can* be preserved by performing a multiplex reaction, since the differences in transcript abundance measured by multiplex PCR were identical to traditional singleplex PCR, but with one quarter of the cDNA input amount, and (3) multiplex PCR is an efficient way to analyze mRNA levels from precious samples.

## Conclusion

Some of the challenges facing researchers are related to obtaining as much genetic information as possible from a small amount of sample. Here, we demonstrate that a new multiplex PCR solution can alleviate some of those challenges. We have shown that multiplex qPCR is as accurate as traditional singleplex or duplex qPCR in two different applications commonly used by research scientists. We have also shown that multiplex qPCR makes more efficient use of samples than singleplex PCR, because the same sample can be efficiently queried with more than one assay at a time. Finally, the same information can be obtained without setting up multiple single-assay reactions, saving time and materials that can reduce the cost associated with complex projects.

## References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10. Cai W, Dong F, Wang Z, et al. (2014) Heated and humidified $CO_2$ pneumoperitoneum inhibits tumour cell proliferation, migration and invasion in colon cancer. *Int J Hyperthermia*, Epub ahead of print.

## Ordering information

| Product | Cat. No. |
|---|---|
| Cell line SW620 | ATCC®: CCL-227™ |
| Cell line Panc 03.27 | ATCC®: CRL-2549™ |
| Cell line MDA-MB-468 | ATCC®: HTB-132™ |
| Human Colon Total RNA | AM7986 |
| TaqMan® Control Genomic DNA® | 4312660 |
| TaqMan® Multiplex Master Mix (5 mL) | 4461882 |
| SuperScript® VILO™ cDNA Synthesis Kit (50 reactions) | 11754050 |
| TaqMan® OpenArray® Real-Time PCR Master Mix (5 mL) | 4462164 |
| TaqMan® Genotyping Master Mix (400 reactions) | 4371355 |
| TaqMan® OpenArray® Human Signal Transduction Panel, QuantStudio® 12K Flex | 4475392 |
| QuantStudio® 12K Flex System with OpenArray™ Block (with AccuFill™ System) | 4471090 |
| QuantStudio® 7 Flex Real-Time PCR System, 96-well, desktop | 4485690 |
| MicroAmp® Optical 96-well Reaction Plate with Barcode (20 plates) | 4306737 |
| MicroAmp® Optical Adhesive Film (100 covers) | 4311971 |

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# TaqMan multiplex real-time PCR

## Get more data out of your sample

- A complete multiplex real-time PCR (qPCR) solution for gene expression and genotyping applications
- Applied Biosystems™ ABY™ and JUN™ dyes, QSY™ quencher, and a multiplex master mix for optimal amplification performance
- Up to 4-plex reactions—as sensitive as singleplex reactions, decreases the starting material required, and minimizes optimization processes

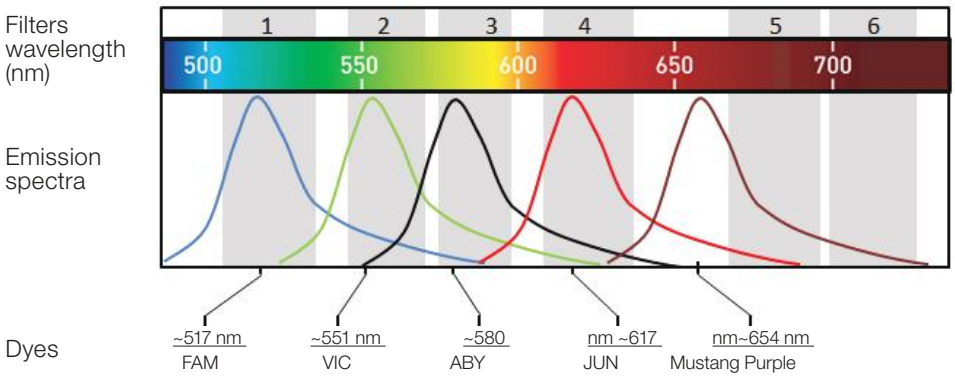


**Figure 1. Fluorescence emission spectra of FAM, VIC, ABY, and JUN dyes used for multiplex real-time PCR.** Grey zones represent the filters available on Applied Biosystems™ real-time PCR systems: 1 through 6 for the QuantStudio™ 7 or 12K Flex Real-Time PCR Systems; 1 through 5 for the QuantStudio™ 6 Flex Real-Time PCR System, ViiA™ 7 Real-Time PCR System, and 7500 or 7500 Fast Real-Time PCR System. MP = Mustang Purple™ dye.

Obtaining the maximum amount of genetic information from an important but small amount of sample can be challenging. This is particularly true with formalin-fixed, paraffin-embedded (FFPE) samples or tumor biopsies that are used for translational research studies. Singleplex qPCR is frequently used for these clinical research samples, but this typically has a higher cost per sample than running in multiplex format. The additional time and materials required to set up multiple single-assay reactions could also significantly increase the cost of a complex project.

Multiplex qPCR, a strategy where more than one target in a sample is amplified and quantified in a single tube, can decrease the quantity of sample material and reagents required. A complete solution for multiplex qPCR is presented here, with components designed to work together for better data quality and less time for optimization. The solution consists of the following:

- Applied Biosystems™ TaqMan® probes using QSY quencher, providing maximal PCR efficiency in a multiplex format. These probes can be ordered with Applied Biosystems™ FAM™ and VIC™ dyes and also with the ABY and JUN dyes, allowing amplification of up to 4 targets in a single reaction. These reporter dyes are optimized to work together with minimal spectral overlap for improved performance (Figure 1). In addition, the QSY quencher is fully compatible with probes that have minor-groove binder (MGB) quenchers.
- The Applied Biosystems™ TaqMan® Multiplex Master Mix was developed to allow amplification of 4 targets simultaneously, without competition between targets. This master mix contains the Applied Biosystems™ Mustang Purple™ dye, a passive reference used for normalization instead of the Applied Biosystems™ ROX™ dye, allowing for measurement of JUN dye in the channel previously used to measure ROX dye.

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- Off-the-shelf, predesigned assays—an RNase P assay using an ABY-QSY probe and a GAPDH assay using a JUN-QSY probe. Both assays are available in limited and nonlimited primer concentrations.
- Calibration plates for ABY, JUN, and Mustang Purple dyes, available in 96-well, 96-well Fast, and 384-well formats.
- Additional services provided through our custom services program—save time and let our Applied Biosystems™ TaqMan® Assay experts design your multiplex assays.

This multiplex solution is compatible with the Applied Biosystems™ QuantStudio™ 6, 7, and 12K Flex Real-Time PCR Systems, as well as the Applied Biosystems™ ViiA™ 7 Real-Time PCR System and the Applied Biosystems™ 7500 and 7500 Fast Real-Time PCR Systems.

## Multiplexing without compromise

The multiplex format enables cost savings and preservation of limited sample, but it's important to obtain the same sensitivity as in the singleplex format. Figure 2 demonstrates comparable results between reactions performed in individual tubes or in 4-plex reactions for a gene quantification experiment.

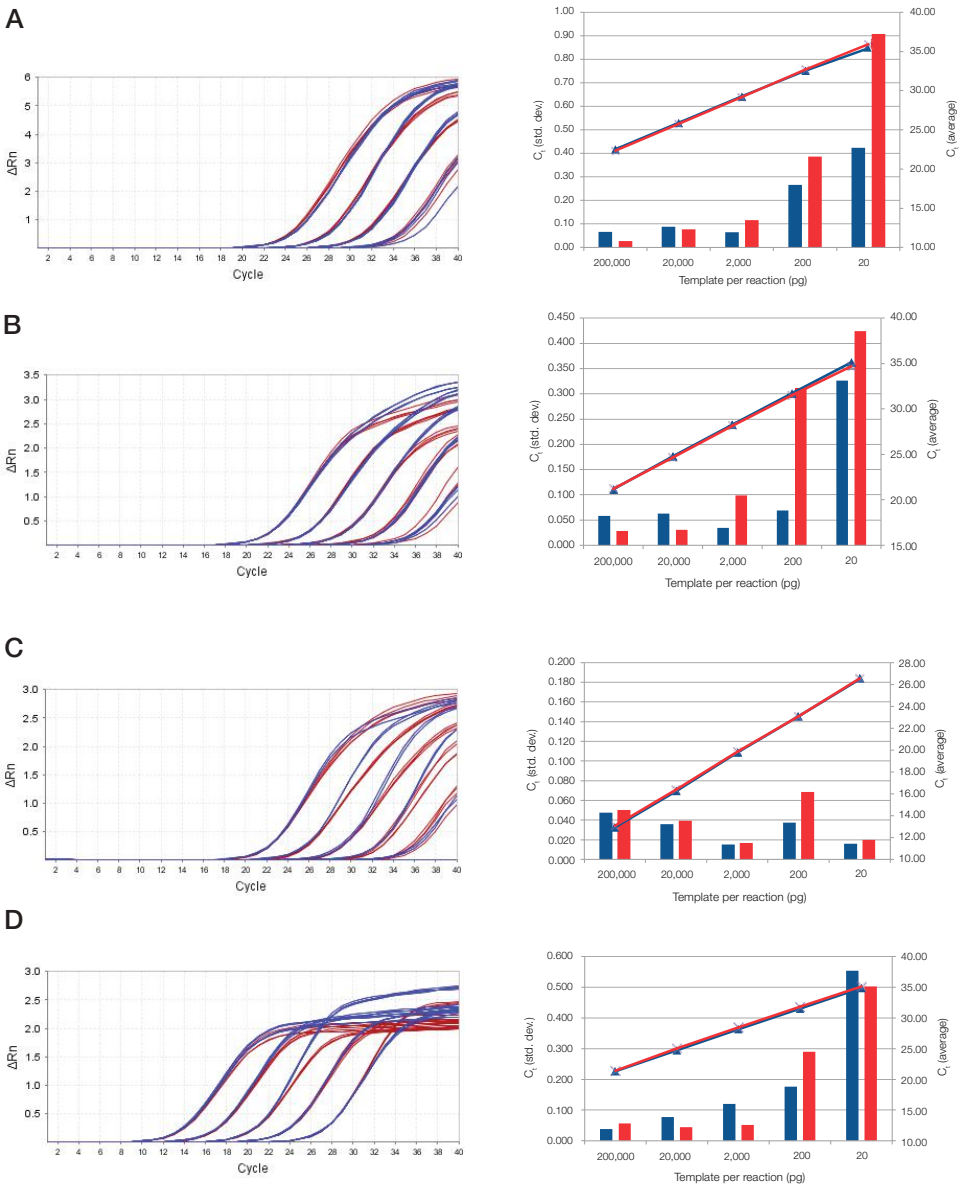


**Figure 2. Comparison of singleplex and multiplex gene expression assays. (A)** EGFR assay, FAM dye; **(B)** BRCA1 assay, VIC dye; **(C)** ESR1 assay, JUN dye; **(D)** RNase P assay, ABY dye. Amplification was performed on the QuantStudio 7 Real-Time PCR System using TaqMan Multiplex Master Mix. The figure shows amplification plots (left) and linear curves (right) for 4 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. Average $C_t$ value (lines) and average standard deviation (bars) for the dilution series are represented in their respective graphs and show the concordance between singleplex and 4-plex reactions. PCR efficiencies are: 96.09% for EGFR singleplex and 96.39% for EGFR 4-plex; 93.56% for BRCA1 singleplex and 94.93% for BRCA1 4-plex; 97.13% for ESR1 singleplex and 95.81% for ESR1 4-plex; 96.91% for RNase P singleplex and 98.1% for RNase P 4-plex.

## Improved probe performance

Introduction of ABY and JUN reporter dyes and Mustang Purple passive reference dye allows for optimal 4-color multiplex assays when used with our FAM and VIC reporter dyes. Please note that ABY and JUN reporter dyes are available only with QSY quencher, while FAM and VIC dyes are available with either MGB or QSY quencher. A comparison with a set of dyes from another supplier shows that our combination of dyes provides an earlier $C_t$ for the majority of assays (Figure 3).

## Optimized multiplex master mix

In multiplex PCR, it's important to have a robust master mix that allows for amplification of each target in a highly competitive environment. Our new master mix composition was developed to provide optimal multiplex performance for each target in the reaction. A comparison of our master mix and a master mix from another supplier in a 4-plex reaction shows an earlier $C_t$ for 3 of the targets amplified with our new master mix and a lower standard deviation for most of the dilution points, demonstrating the excellent performance of our solution (Figure 4).

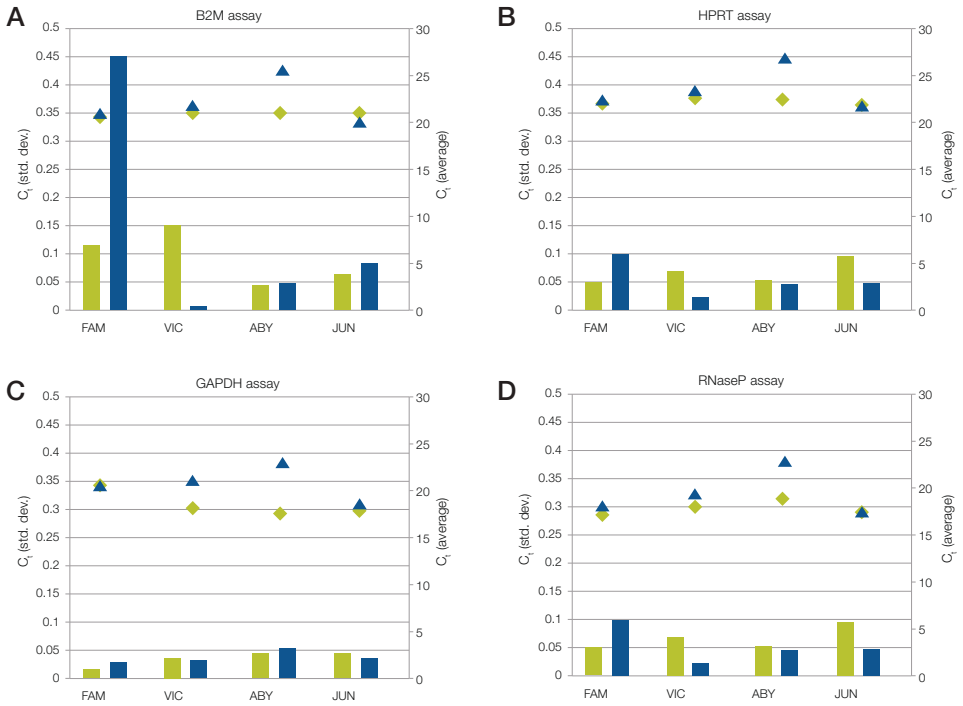


**Figure 3. Comparison of our new dye combination with a dye combination from another supplier.** Probes for **(A)** B2M, **(B)** HPRT, **(C)** GAPDH, and **(D)** RNase P gene expression assays were synthesized with FAM, VIC, ABY, and JUN dyes with QSY quencher (green bars and diamonds) and with another commercially available dye combination (blue bars and triangles). All possible gene-dye combinations were tested. Reactions were prepared with TaqMan Multiplex Master Mix using 900 nM of primer, 250 nM of probe, and 10 ng of cDNA. Amplification was performed on the QuantStudio 7 Real-Time PCR System using TaqMan Multiplex Master Mix. Bars represent average standard deviation. Triangles and diamonds represent average $C_t$ values.

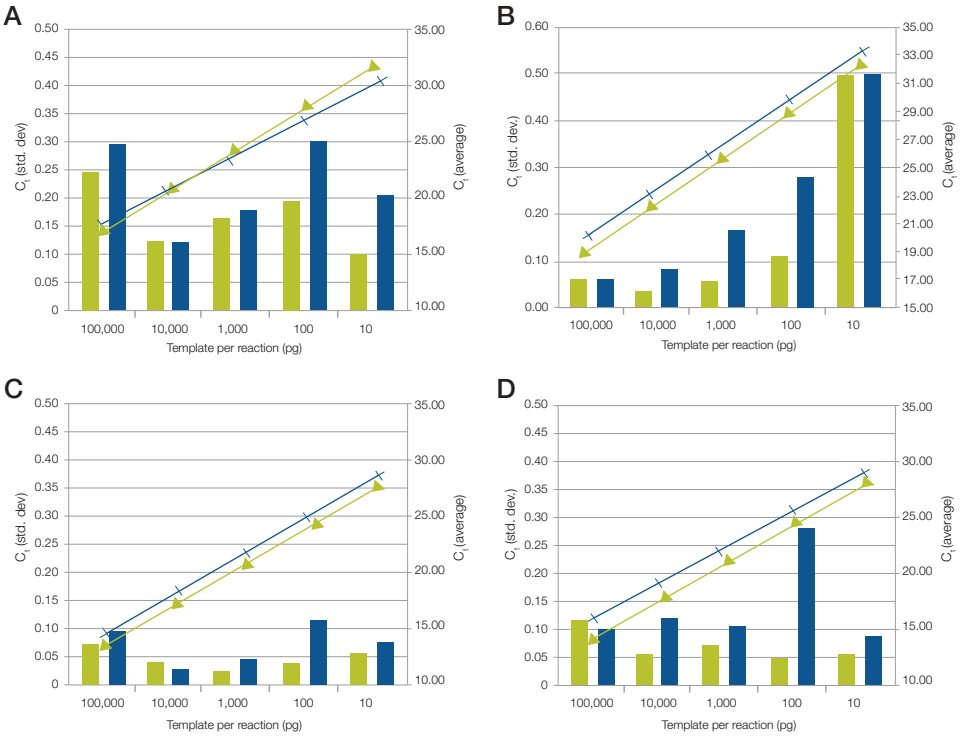


**Figure 4. Comparison of TaqMan Multiplex Master Mix with another commercially available master mix. (A)** B2M assay, FAM dye; **(B)** RNase P assay, VIC dye; **(C)** GAPDH assay, ABY dye; **(D)** HPRT assay, JUN dye. All assays used QSY quencher. The graph shows average standard deviation (bars) and average $C_t$ values (cross and triangle) for 4-plex reactions using a dilution series from 100 ng to 10 pg of cDNA per 10 µL reaction. All amplifications were performed on the ViiA 7 Real-Time PCR System using the cycling conditions recommended for each master mix. Green represents TaqMan Multiplex Master Mix, and blue represents 4-plex reactions with another commercially available master mix.

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## Optimized to minimize time-to-results

Developing a multiplex PCR assay requires time to correctly design the assay and optimize the reaction. Using our complete solution, for which all components were developed to work together, helps increase your chances of success and limits your development time. A new multiplex PCR user guide was developed to guide you through the development and optimization process [1], and our custom services will allow you to delegate assay design to our experienced team to minimize your efforts.


### References

1. Multiplex PCR User Guide. Available at thermofisher.com/multiplexqpcr
2. TaqMan multiplex qPCR: Accurate, sensitive, and as efficient as traditional singleplex qPCR. Application note available at lifetechnologies.com/multiplexqpcr


### Ordering information

| Product | Cat. No. |
|---|---|
| **TaqMan QSY probes** | |
| TaqMan QSY Probe, 6,000 pmol | 4482777 |
| TaqMan QSY Probe, 20,000 pmol | 4482778 |
| TaqMan QSY Probe, 50,000 pmol | 4482779 |
| **Control kits** | |
| TaqMan GAPDH Assay, JUN-QSY 20X | 4485712 |
| TaqMan GAPDH Assay, JUN-QSY PL 20X | 4485713 |
| TaqMan RNaseP Assay, ABY-QSY 20X | 4485714 |
| TaqMan RNaseP Assay, ABY-QSY PL 20X | 4485715 |
| **Multiplex master mixes** | |
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| TaqMan Multiplex Master Mix, 5 mL | 4461882 |
| TaqMan Multiplex Master Mix, 50 mL | 4486295 |

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| | |
|---|---|
| **Calibration plates** | |
| 96-Well Calibration Plate, Mustang Purple dye | 4461599 |
| 96-Well Calibration Plate, JUN dye | A24737 |
| 96-Well Calibration Plate, ABY dye | A24738 |

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# PowerTrack SYBR Green Master Mix

## Easy-to-use and flexible gene expression master mix for real-time PCR

Applied Biosystems™ PowerTrack™ SYBR Green Master Mix is a preformulated, optimized, universal 2X master mix for real-time PCR. Building on over 25 years of innovation and product excellence in qPCR, our PowerTrack SYBR Green Master Mix is designed for superior performance and ease of use with a two-color tracking dye system for the most common real-time PCR applications.

### Features include:

- Built-in two-color tracking dye system where pipetting has occurred
- Broad primer $T_m$ and primer concentration compatibility allows flexibility in qPCR reaction setup with minimal optimization
- Superior specificity and tight reproducibility in $C_t$ values over a broad dynamic range improve data quality
- Compatible with Invitrogen™ SuperScript™ IV VILO™ Master Mix reverse transcription for fast, reproducible results
- Formulated with UNG and dUTP to prevent contamination of downstream reactions by carryover PCR products
- Broad instrument compatibility

### Built-in visual indicator to aid in reaction setup

PowerTrack SYBR Green Master Mix is designed to provide ease in visualization of sample addition to the master mix. The master mix contains an inert blue dye and a separate, optional yellow sample buffer. The yellow sample buffer is added separately to indicate that sample has been added to the reaction, based on a visual color change of the reaction mix from blue to green. The benefit of using the tracking dye is to provide convenience via visualization of the color change chemistry and avoid errors that can occur due to pipetting mistakes. The yellow sample buffer is provided to aid in reaction setup for your own peace of mind but is not required to obtain superior results with PowerTrack SYBR Green Master Mix.

### Formulated for maximum specificity and reproducibility

PowerTrack SYBR Green Master Mix uses an antibody-mediated hot-start mechanism to provide tight control over *Taq* enzyme activation and help prevent early activity of the polymerase at low temperatures that can lead to nonspecific amplification.

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## High specificity

In an evaluation of 24 different primer sets used with PowerTrack SYBR Green Master Mix, a single melt curve was obtained in 100% of reactions. In contrast, nonspecific amplification was observed for some of the same targets with several master mixes from other suppliers, as shown by multiple peaks in the melt curves (Figure 1). Verification of primer specificity in SYBR Green reactions is essential to data quality and validity [1]. The high specificity enabled by PowerTrack SYBR Green Master Mix allows you to spend less time optimizing and redesigning primers to get high-quality data.


**Figure 1. Target specificity.** Real-time PCR was performed using universal human reference (UHR) cDNA and primers targeting *PGK1* (phosphoglycerate kinase 1), *ARL1* (ADP-ribosylation factor-like protein 1), and *SNF8* (vacuolar-sorting protein). Reactions (10 µL) were run in quadruplicate using the indicated master mixes on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System. Several master mixes from other suppliers show a second peak in the melt curve analysis attributed to amplification of nonspecific product.

## PowerTrack SYBR Green Master Mix powers through traditionally difficult targets

Amplification curves were obtained for *PGK1* over a 6-log dilution series of UHR cDNA. PowerTrack SYBR Green Master Mix delivers accurate results over a wide dynamic range of concentrations as shown by tight curves between replicates and superior PCR efficiency (Figure 2).*

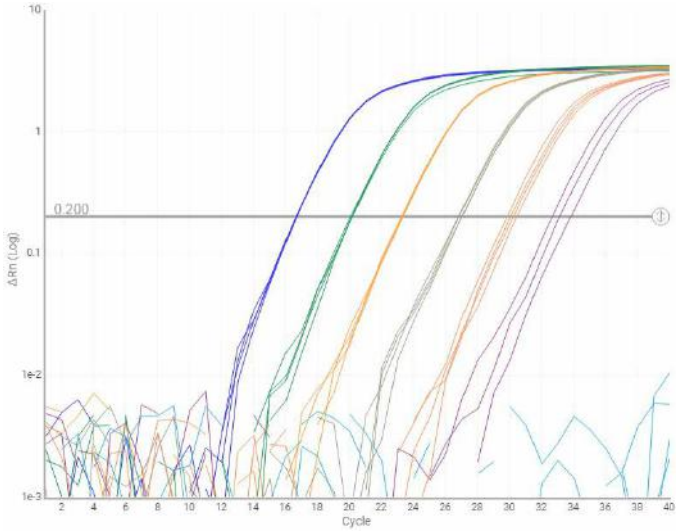


**Figure 2. Linear dynamic range.** PowerTrack SYBR Green Master Mix enables reliable results across a range of cDNA concentrations. Amplification curves were obtained for *PGK1* over a dilution series (10 ng to 100 fg and no template control (NTC)) of UHR cDNA. Reactions were run in quadruplicate on the QuantStudio 5 Real-Time PCR System using 60ºC as the annealing $T_m$ for primers.

## Excellent reproducibility

Reproducibility is another important measure of data quality in real-time PCR, and reproducibility is often affected at low template concentrations, where the effects of variability are exacerbated. However, PowerTrack SYBR Green Master Mix demonstrated excellent reproducibility over a wide dynamic range with a variety of targets and reverse transcription (RT) kits tested (Figure 3). Tighter reproducibility allows for greater statistical significance when analyzing low-abundance transcripts and smaller fold changes.

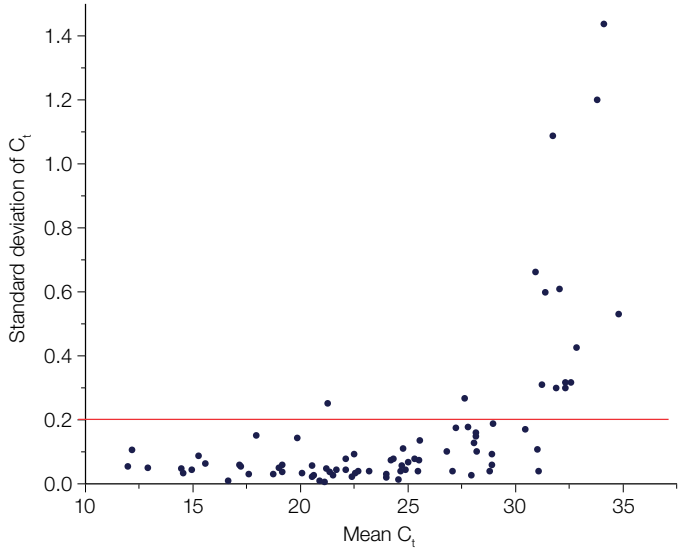


**Figure 3. Reproducibility of data.** PowerTrack SYBR Green Master Mix shows reproducibility over a wide dynamic range. Six assays (PGK1, ARL1, SNF8, DF, GAPDH, and Corf1 19) were run in quadruplicate with UHR cDNA generated from four different RT kits (SuperScript IV VILO Master Mix, Applied Biosystems™ High-Capacity RNA-to-cDNA Kit, iScript™ cDNA Synthesis Kit, and QuantiTect™ RT Kit) run with a 6-fold dilution series and 400 nM primer concentration. Assays were performed on the QuantStudio 5 Real-Time PCR System.

* Besides the master mix, other assay conditions and reagent concentrations may affect dynamic range; individual results may vary.

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## Broad instrument compatibility

PowerTrack SYBR Green Master Mix can be used in either standard or fast cycling mode and is compatible with all Applied Biosystems™ real-time PCR instruments. It is also compatible with the Bio-Rad CFX96™, CFX384™, and iQ™5 instruments, as well as the Roche LightCycler™ 480 and Agilent Mx3005P™ instruments.

## Why ROX dye matters

ROX™ dye is an inert reference dye used in RT-qPCR, often added to a master mix. It is effective in normalizing fluorescence across all samples. ROX dye removes fluorescence variations, such as those caused by bubbles in the reactions. Applied Biosystems™ master mixes contain a proprietary ROX dye, specifically formulated for a wide range of PCR instruments and for compatibility with a wide range of differing instrument light sources and filter sets. Most other manufacturers use a ROX dye that contains only a single excitation peak. These manufacturers may require a ROX dye to be spiked into the reaction at a concentration appropriate to the instrument. Alternatively, they may require selection of either a "low ROX" or "high ROX" master mix, depending on the concentration.

## Heat-labile UNG for carryover contamination control

Contamination is a major concern in labs that routinely run PCR due to the potential for false-positive results. The inclusion of UNG and dUTP in the PowerTrack SYBR Green Master Mix allows any previously amplified PCR products to be degraded and helps prevent contamination of subsequent qPCR reactions.


**Reference**

1. Bustin SA, Benes V, Garson JA et al. (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* 55:611–622.


**Ordering information**

| Product | Quantity | Cat. No. |
|---|---|---|
| PowerTrack SYBR Green Master Mix, Mini Pack (1 mL) | 100 reactions | A46012 |
| PowerTrack SYBR Green Master Mix, 1-Pack (1 x 5 mL) | 500 reactions | A46109 |
| PowerTrack SYBR Green Master Mix, 2-Pack (2 x 5 mL) | 1,000 reactions | A46110 |
| PowerTrack SYBR Green Master Mix, 5-Pack (5 x 5 mL) | 2,500 reactions | A46111 |
| PowerTrack SYBR Green Master Mix, 10-Pack (10 x 5 mL) | 5,000 reactions | A46112 |
| PowerTrack SYBR Green Master Mix, Bulk Pack (1 x 50 mL) | 5,000 reactions | A46113 |

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

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**COL33396 0220**

# PowerTrack™ SYBR™ Green Master Mix

Master mix with a two-dye tracking system for real-time PCR workflows

Catalog Numbers A46012, A46109, A46110, A46111, A46112, A46113

Pub. No. MAN0018826 Rev. B.0

**Note:** For safety and biohazard guidelines, see the "Safety" appendix in the *PowerTrack™ SYBR™ Green Master Mix User Guide* (Pub. No. MAN0018825). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This Quick Reference is intended as a benchtop reference for experienced users of PowerTrack™ SYBR™ Green Master Mix. For detailed instructions, supplemental procedures, and troubleshooting, refer to the *PowerTrack™ SYBR™ Green Master Mix User Guide* (Pub. No. MAN0018825).

## Guidelines

### Requirements for input DNA

Use 1–10 ng of cDNA or 10–100 ng of gDNA per reaction.

### Guidelines for PCR reactions

- Four replicates of each reaction are recommended.
- Reaction mixes can be prepared depending upon experimental requirements. Scale the components according to the number of reactions and include 10% overage.
- If using smaller reaction volumes, scale all components proportionally. Reaction volumes <10 µL are not recommended.
- The recommended final primer concentration for primers with a Tm of 55°C is 400 nM.

### Guidelines for no-template control reactions

No-template control (NTC) reactions can be used to identify PCR contamination. NTC reactions contain all of the reaction components except for the sample.

## Methods

### Set up the plate document or plate file

Configure the plate document or plate file.

See the appropriate instrument user guide for detailed instructions.

### Prepare the reagents

- Thaw the master mix.
- Once the master mix is thawed, swirl it to mix thoroughly.
- Thaw the DNA samples and primers on ice, vortex to mix, then centrifuge briefly.
- Vortex the Yellow Sample Buffer prior to use.

### Prepare the PCR reactions

**Note:** The Yellow Sample Buffer is optional for the real-time PCR.

The Yellow Sample Buffer is supplied at a 40X concentration. It is added to the DNA template. The concentration of Yellow Sample Buffer in the final PCR must be 1X. It is recommended that the DNA template is 10–20% of the volume of the final PCR.

1. *(Optional)* Add the Yellow Sample Buffer (40X) to the amount of DNA that is used in the PCR.

| Final reaction volume | Amount of Yellow Sample Buffer |
|---|---|
| 20 µL | 0.5 µL |
| 10 µL | 0.25 µL |

The Yellow Sample Buffer is diluted to 1X in the final reaction. See the following tables.

2. *(Optional)* Vortex, then centrifuge the DNA and Yellow Sample Buffer.

3. Combine the master mix, the primers, and nuclease-free water according to the following tables.

4. Combine the master mix, the primers, and nuclease-free water with the DNA and Yellow Sample Buffer according to the following tables.

**Note:** If the Yellow Sample Buffer is not used, add nuclease-free water to achieve the total PCR volume.

**Table 1 20–µL reaction**

| Component | Stock concentration | Final concentration | Volume for 1 reaction (20–µL reaction) | Volume for 4 reactions with 10% overage (20–µL reaction)[1] |
|---|---|---|---|---|
| **Yellow Sample Buffer and DNA (step 1)** | | | | |
| DNA[2] | 5 ng/µL | 0.5 ng/µL | 2 µL[3] | 8.8 µL |
| Yellow Sample Buffer | 40X | 1X | 0.5 µL | 2.2 µL |
| **Master mix, primers, and nuclease-free water (step 3)** | | | | |
| PowerTrack™ SYBR™ Green Master Mix | 2X | 1X | 10 µL | 44.0 µL |
| Forward and reverse primers[4] | 8,000 nM | 400 nM | 1 µL | 4.4 µL |
| Nuclease-free water | — | — | 6.5 µL | 28.6 µL |
| **Total PCR volume** | **—** | **—** | **20 µL** | **88 µL** |

[1] 10% overage is recommended for pipetting variations.
[2] Use 1–10ng of cDNA.
[3] Does not exceed 8.5 µL.
[4] The final primer concentration can vary from 300–800 nM. A final concentration of 400 nM is recommended for primers with a Tm of 55°C.

**Table 2 10–µL reaction**

| Component | Stock concentration | Final concentration | Volume for 1 reaction (10–µL reaction) | Volume for 4 reactions with 10% overage (10–µL reaction)[1] |
|---|---|---|---|---|
| **Yellow Sample Buffer and DNA (step 1)** | | | | |
| DNA[2] | 5 ng/µL | 0.5 ng/µL | 1 µL[3] | 4.4 µL |
| Yellow Sample Buffer | 40X | 1X | 0.25 µL | 1.1 µL |
| **Master mix, primers, and nuclease-free water (step 3)** | | | | |
| PowerTrack™ SYBR™ Green Master Mix | 2X | 1X | 5 µL | 22.0 µL |
| Forward and reverse primers[4] | 8,000 nM | 400 nM | 0.5 µL | 2.2 µL |
| Nuclease-free water | — | — | 3.25 µL | 14.3 µL |
| **Total PCR volume** | **—** | **—** | **10 µL** | **44 µL** |

[1] 10% overage is recommended for pipetting variations.
[2] Use 1–10ng of cDNA.
[3] Does not exceed 4.25 µL.
[4] The final primer concentration can vary from 300–800 nM. A final concentration of 400 nM is recommended for primers with a Tm of 55°C.

**IMPORTANT!** The reaction turns green due to the Yellow Sample Buffer added to the DNA and the inert blue dye in the master mix.

5. Mix the components thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.

6. Transfer the appropriate volume of each reaction to each well of an optical plate.

7. Seal the plate with an optical adhesive cover, then centrifuge briefly to collect the contents at the bottom of each well and eliminate any air bubbles.

PCR can be performed on the reaction plate up to 8 hours after completing the set-up, when stored at room temperature protected from light.

## Set up and run the real-time PCR instrument

1. Set up the thermal protocol according to one of the following tables.

   **Note:** Standard cycling conditions are recommended for genomic DNA templates or long amplicons.

   **Table 3 Fast cycling mode**

| Step | Temperature | Duration | Cycles |
|---|---|---|---|
| Enzyme activation | 95°C | 2 minutes | 1 |
| Denature | 95°C | 5 seconds | 40 |
| Anneal/extend | 60°C | 30 seconds | |

   **Table 4 Standard cycling mode**

| Step | Temperature | Duration | Cycles |
|---|---|---|---|
| Enzyme activation | 95°C | 2 minutes | 1 |
| Denature | 95°C | 15 seconds | 40 |
| Anneal/extend | 60°C | 60 seconds | |

2. Set the instrument to perform a default dissociation step, according to one of the following tables.

   **Table 5 Fast cycling mode**

| Step | Ramp rate[1] | Temperature | Time |
|---|---|---|---|
| 1 | 1.99°C/second | 95°C | 15 seconds |
| 2 | 1.77°C/second | 60°C | 1 minute |
| 3 (Dissociation) | 0.075°C/second | 95°C | 15 seconds |

[1] Use the default ramp rate for the StepOnePlus™ Instrument.

   **Table 6 Standard cycling mode**

| Step | Ramp rate[1] | Temperature | Time |
|---|---|---|---|
| 1 | 1.6°C/second | 95°C | 15 seconds |
| 2 | 1.6°C/second | 60°C | 1 minute |
| 3 (Dissociation) | 0.075°C/second | 95°C | 15 seconds |

[1] Use the default ramp rate for the StepOnePlus™ Instrument.

   **Note:** A dissociation step must be performed immediately after the real-time PCR run with PowerTrack™ SYBR™ Green Master Mix.

3. Set up the options.
   - Experiment type: Standard curve
   - Reagent: SYBR™ Green reagents
   - Reporter: SYBR™ Green
   - Quencher: None
   - Passive reference dye: ROX™ dye
   - Ramp speed: Standard or fast
   - Melt curve ramp increment (all instruments, except StepOnePlus™ instrument): Continuous

*(StepOnePlus™ only):* Step and hold

4. Set the reaction volume appropriate for the reaction plate.
5. Load the reaction plate into the real-time PCR instrument.
6. Start the run.

## Analyze the results

1. View the amplification plots.
2. Determine the baseline and threshold cycles ($C_q$) for the amplification curves using the instrument software.
3. Check for nonspecific amplification using melt curves.
4. Perform relative or absolute quantitation.

| Option | Description |
|---|---|
| Relative quantitation | The target is compared to an internal standard, using either the standard curve or comparative $C_q$ method. |
| Absolute quantitation | The $C_q$ of the unknown samples is compared against a standard curve with known copy numbers. |

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.



**Revision history:** Pub. No. MAN0018826

| Revision | Date | Description |
|---|---|---|
| B.0 | 29 July 2022 | The volumes for preparing the PCR reactions were corrected (Table 1 on page 2 and Table 2 on page 2). |
| A.0 | 30 January 2020 | New document. |