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Instruction for use Use by professionals only Tests per ml: max. 20	CE 0483
Revision:	29/07-2019
Product-Name:	**Product-Code:**
Anti-D blend TH-28/MS26	D-mono-blend-TH
Anti-D blend 175 2-415 1E4	D-mono-blend-175
monoclonal (human IgM/IgG)	

Reagent for specific detection of D-antigen. Bloodgroup testreagent for microplates-, slide-, plate- or tube-, as well as indirect antiglobulin techniques.
All described test methods are only valid for manual applications as recommended in this instruction. The user must determine their suitability for use in other techniques (automates, semi-automates, gel-cards, others) according to recognized techniques in individual responsibility.
Only for in-vitro diagnostic laboratory use. Store at + 2 - 8 °C when not in use.

Intended use:	Anti-D is a monoclonal human IgM/IgG blood grouping reagent (cell lines TH-28/MS-26 or 175 2-415 1E4) which detects antigen when tested according to the microplate-, slide-, plate- or tube- as well as the indirect antiglobulin techniques.
Introduction:	**The Rh Blood Group System:** The observations of Levine and Stetson in 1939 and of Landsteiner and Weiner in 1940 provided the basis for current understanding of the clinical significance and laboratory detection of Anti-D. Approximately 15% of Caucasians lack the RhD antigen and are easily stimulated by a RhD positive pregnancy or blood transfusion to produce Anti-D. This may cause haemolytic disease of the newborn or severe haemolytic transfusion reactions.
Weakened Expression of the RhD antigen:	The collective term D^u is widely usede to describe red cells which have a weaker expression of the D antigen than normal. The term D weak denotes individuals with a reduced number of entire D antigen sites per red cell. The term D partial denotes individuals with missing D epitopes. D category VI is the D partial category which lacks most D epitopes. This reagent will detect partial D´s including D category VI cells (as required by the UKBTS and BCSH Guidelines). This reagent is ideal for patient and donor testing.
Principle of the reagent:	Anti-D monoclonal human IgM/IgG blood grouping reagent contains antibodies from cell lines TH-28/MS-26 or 175 2-415 1E4. When used by the recommended techniques this reagent will cause agglutination (clumping) of red cells carrying the specific antigen (positive test). Lack of agglutination of the red cells demonstrates the absence of the specific antigen (negative test). The reagent has been optimised for use by the recommended techniques without further dilution or additions. The product is supplied sterile filtered to 0.22um.
Material:	Anti-D (cell lines TH-28/MS-26 or 175 2-415 1E4) blood grouping reagent is composed of monoclonal human IgM/IgG antibodies in a buffer solution containing macromolecular chemical potentiators. The reagent contains < 0,1% (w/v) sodium azide and bovine material. Each vial (10ml) contains sufficient material for approximately 250 tests.
Precautions:	1. All blood products should be treated as potentially infectious. The human donor or the cell line used to produce this reagent has been tested and found to be negative for Anti-HIV, Anti-HCV, HbsAg, EBV and Mouse Antibody Production (MAP) viruses. No known tests can guarantee that any product derived from human blood is free from infectious agents. Car must be taken in the use and disposal of each container and its contents. 2. The reagent contains < 0.1% (w/v) sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form highly explosive salts. On disposal, flush with large quantities of water. 3. This product should be clear.Turbidity may indicate bacterial contamination. The reagent should not be used if a precipitate, fibrin gel or particles are present. 4. The reagent is for professional in vitro diagnostic use only. 5. This reagent contains bovine material obtained from a USDA approved source free of Transmissable Spongiform Encephalopathies (TSEs).
Advice to users:	It is recommended that a positive control and a negative control should be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show the expected reactions. It is not required to use a reagent control in parallel with all tests using this reagent. Only in typing the red cells of patients known to have auto antibodies or protein abnormalities is the use of a reagent control recommended. This should be tested in parallel with the reagent. The reagent has been characterised by the procedure recommended in this package insert, its suitability for use in other techniques must be determined by the user. In the event of changes in the analytical performance of the device or damage to the packaging please contact the Quality Assurance department at CE-IMMUNDIAGNOSTIKA GmbH.
Storage:	Store the opened/unopened product at 2-8°C until the expiry date detailed on the product label. Failure to store the product at the correct temperature, for example, storage at higher temperatures or repeated freezing and thawing may result in accelerated loss of reagent activity
Specimen collection:	No special preparation of the patient is required prior to specimen collection. Blood should be collected by an approved phlebotomy technique. The proof of the RhD antigen should be tested as soon as possible following collection. If a delay in testing should occur, store the specimen at 2-8°C. Specimens displaying gross haemolysis or microbial contamination should not be tested with this reagent. Failure to store the specimens at the correct temperature, for example, storage at higher temperature or repeated freezing and thawing may result in false positive or false nagative results.
Material required but not provided:	**Slide technique or Plate test:** Microscope slides or plates, sticks and pipettes. **Tube technique:** Test tube, Pipettes, Centrifuge (1000 crf), Isotonic saline, timer, 37°C incubator **Indirect Antiglobulin technique for detection of Dweak:** Test tubes, pipettes, 37°C incubator, Anti-Human-Globulin reagent, Centrifuge (1000 crf), Coombs control cells, Isotonic saline, Timer. **Microplate technique:** Microplates, Pipettes, Mikroplate shaker, Centrifuge (1000 crf), automated microplate reader (optional), Isotonic saline, Timer.

Recommended Techniques:

Slide-test / plate test:

1.	Slide-tests are performed with whole blood, plate-tests with 10% Erythrocytes suspension in NaCl solution or who It is generally recommended to wash patients or donors cells before use.
2.	Place 1 drop of Anti-D blend reagent (appr. 40µl) on a clean glass-, plastic slide or plate.
3.	Slide techniques: Add 1 drop of whole blood (about 35-45% suspension of red cells) or Plate technique: Add 1 drop of whole blood respectively 10% red cell suspension in 0,9% saline solution.
4.	On glass slides, use a separate clean applicator stick to mix each reagent/cell mixture over an area approximately 20mm diameter. On plastic slides follow the manufacturer's insert.
5.	Observe for macroscopic agglutination and record results. This is achieved on glass slides by slow rotation over a period up to 2 minutes and on plates after an incubation time of 5-10 minutes. Care should be taken not to mistake peripheral drying or fibrin strands as agglutination. Do not place the slides or plates on a heated illuminated surface.

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Tube technique:	1. Prepare a 2-5% Erythrocytes suspension of test red cells in isotonic saline. (The cells should have been washed at least once.) 2. Add 1 drop Anti-D reagent to an appropriately labelled test tube. 3. Add 1 drop of the suspension of test red cells. 4. Mix and centrifuge for 1 min. at 400 rcf. (about 1500 UpM) or for 20 seconds at 1.000 x g or at appropriate time and force to produce the strongest reactions. 5. Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination. 6. Incubate all negative or weakly positive tests at 37°C for 5 minutes and repeat stages 4 and 5. This may enhance the reaction strength in typing red cells of rare phenotypes. Record results.
Indirect Antiglobulin technique for weakened expression of the D Antigen:	1. Prepare a 2-5% Erythrocytes suspension of test red cells in isotonic saline (The cells should have been washed at least once.) 2. Add 1 drop Anti-D reagent to an appropriately labelled test tube. 3. Add 1 drop of the suspension of test red cells. 4. Mix and incubate at 37°C for 15 minutes. 5. Wash the cells 3x with isotonic saline, thoroughly decanting the saline. 6. Add 2 drops of Anti-Human Globulin reagent, mix and centrifuge for 1 min. at 400 rcf. (about 1.500 UpM) or for 20 seconds at 1.000 x g or at appropriate time and force to produce the strongest reactions. 7. Gently agitate the tube to dislodge the red cells. Examine macroscopically for agglutination. Agglutinations prove the presence of Dweak cells. 8. To confirm that negative tests are valid, add IgG sensitised red cells (Coombs control cells), repeat centrifugation and examine for agglutination. If no agglutination is observed the test is invalid and should be repeated.
Microplate technique:	1. Prepare a 2-5 % Erythrocytes suspension of test red cells in isotonic solution. (Recommendation 2% suspension) (The cells should have been washed at least once.) 2. Add 1 drop Anti-D reagent (30-50µl) to the appropriate test wells of a U well microplate. 3. Add an equal volume of the cell suspension to the appropriate test wells. 4. Mix the contents of each well using manual means or a microplate shaker. (30 sec.) 5. Cell line TH-28/MS26: incubate 15- 20 min. at RT, centrifuge 40 sec. at 100 rcf . or at other appropriate time and UpM. Cell line 175 2-415 1E4: centrifuge without incubation time 20 - 30 sec. at 1.000 crf. or at other appropriate time and UpM. 6. Shake MTP manually, if necessary with a shaker. 7. Read tests macroscopically or with an automated plate reader, record results. The use of an automated plate reader must be validated by the customer. The use of additional visual remedies as mirror or magnifier can ease the reading.
Limitations:	Rarely in-vivo loaded red cells may produce false positive results in a direct antiglobulin test (DAT). The use of CE-IMMUNDIAGNOSTIKAs monoclonal Control reagent is recommended for detection of such potentially false positive results. Rigid polystyrol microplates are generally more suitable than those made from PVC. Each batch of microplates should be evaluated in the user´s system prior to acceptance as suitable for routine usage. False positive or false negative results may occur through contamination of test materials or any deviation from the recommended technique.
Performance Characteristics:	Anti-D blend (cell line TH-28/MS-26 or 175 2-415 1E4) monoclonal human IgM/IgG blood grouping reagent product has been tested by each of the recommended techniques with donor, clinical and neonatal specimens. These were collected in either the anticoagulant EDTA, CPDA or ACD. The sample population represented all mayor RhD phenotypes.
Performance data:	The reagent fulfils the common technical specifications' requirements according to Annex II, List A der Directive 98/79/EC for in vitro diagnostics. It has the same or a better performance characteristics as comparable reagents in use. It was tested on more than 1000 samples with sensitivity and specificity of 100%.
References:	1. Widmann F.K.ed Technical Manual 10th Ed Washington DC, American Association of Blood Banks 1990, Chapter 11. 2. Race R.R. and Sanger R.Blood Groups in Man, 6th Edition Oxford Blackwell Scientific Publishers 1975:178 3. Issit P.D.and Anstee, D.J.Applied Blood Group Serology 4th Edition, Montgomery Scientific Publications, Miami, Florida, USA, 1998, Chapter 12. 4. Guidelines for the Blood Transfusion Services in the UK 5th Edition 2001. The Stationary Box.