TransClone® Anti-RH1(D) FAST M
86370 (10 mL) 86371 (10 x 10 mL)
86893 (100 x 10 mL) 86891 (10 L)
86889 (1 L)

HUMAN MONOCLONAL IgM ANTIBODY
RH1 Ag determination
SLIDE (+15°C - +25°C) TUBE (IMMEDIATE SPIN)
AND MICROPLATE (+15°C - +25°C)

All the products manufactured and commercialised by Bio-Rad are under complete quality system starting from reception of raw material to the final commercialisation of the product. Each lot is submitted to a quality control and only is released on the market when conforming to the acceptance criteria. The records relating to production and control of each single lot are kept within our company.
I - USE AND PRINCIPLE OF THE TEST
This reagent (serum-test) is strictly reserved for professional and in vitro diagnostic use.
Designed for the determination of RH1 (D) antigen, the test is based on the principle of agglutination.
Human red blood cells possessing RH1 (D) antigen will agglutinate in the presence of the TransClone Anti-RH1 (D) FAST M reagent, whereas red blood cells lacking RH1 (D) antigen will not agglutinate.
The test can be carried out by using one of the three following techniques: slide (+15°C - +25°C), tube (immediate spin) or microplate (+15°C - +25°C).

II - CHARACTERISTICS OF THE REAGENT
This reagent is supplied in liquid form, ready for use, provided with a dropper (one drop = 50 μl).
The anti-RH1(D) monoclonal antibody used as the active fraction of this reagent, is obtained from the heterohybride B9A4 - B2A6A6A1A1. This clone produces IgM class antibodies.
This reagent contains sodium azide (< 0.1%) as a preservative. Sodium azide may react with laboratory plumbing into copper or lead azides. Such azides are explosive. To prevent azide build-up, flush the pipes with a large quantity of water if solutions containing azide are thrown in the sink after inactivation.
The code number and the volume are mentioned on the box label.

III - STORAGE – SHELF LIFE
The expiry date and storage conditions are stated on the vial’s label.
Store the reagent at +2°C - +8°C.
After opening, subject to storage at +2°C to +8°C and by avoiding contamination, the reagent is stable until the expiry date indicated on the vial.

IV - WARNINGS AND PRECAUTIONS
The reliability of the results depends on correct adherence to the following Good Laboratory Practices:
• Do not use the reagents after the expiration date indicated on the label.
• Do not mix reagents from different lots in a same batch.
• It is essential to take precautions in order not to provoke contamination, particularly during the distribution steps.
• Use a different tip for each sample.
• Check that the pipettes and other apparatus are working correctly and check their precision.
• Wear gloves and safety glasses when handling the reagents and samples.
• Never pipette directly by mouth.
• Handle any specimen as if capable of transmitting disease.
• Avoid splashing. In the event of splashes, clean with 12°C Cl bleach (Javel water) diluted 1:10 and wipe with absorbent paper. The materials used for cleaning are to be discarded in the contaminated waste container.

• Consumables and products which have been in contact with either samples or reagents which contain material of human origin, must be discarded after they have been decontaminated.

• The safety datasheet is available on request.

V - SAMPLING AND SAMPLE PROCESSING
Draw the blood aseptically into a tube with anticoagulant (EDTA, CPD). Conduct the test as soon as possible after sampling. Samples that cannot be analysed rapidly should be stored between +2°C and +8°C and tested within 48 hours. Under no circumstance should haemolysis be visible. Do not heat the samples.

VI - METHOD

Equipment supplied
• TransClone Anti-RH1 (D) FAST M

Material required but not provided
• Isotonic saline solution (0.85-0.90% NaCl)
• Microdil
• Microbrom
• RH Control M
• Centrifuge
• Automatic or semi-automatic pipettes
• Pipette tips
• Disposable tubes
• Glass rod
• Opaline slide
• U shaped microplates
• Microplate shaker
• Microplate centrifuge
• Container for wastes associated with a biological risk
• Bleach (Javel water)
• Latex gloves
• Absorbent paper
• Safety glasses

Controls
• RH Control M : low protein medium, highlights a possible non-specific agglutination due to the reactional medium. Taking into account the weak protein rate of the monoclonal reagents IgM of the TransClone range, the use of RH Control M is not essential in tube and slide techniques but can nevertheless be required by certain authorities. It is, on the other hand, required in microplate technique.
- Positive and negative controls: red blood cells known to be positive and negative for the RH1 (D) antigen are tested together with the sample to validate the reagent activity.

**Procedure**

**Strictly comply with the procedure.**

Allow all the reagents to reach room temperature before use.

Separate the plasma from the red blood cells of the sample by centrifuging (2000 g x 2 minutes).

**A - Slide method (+15°C - +25°C)**

1. On a slide, at room temperature (+15°C - +25°C), deposit 70 μl of the reagent.
2. Add 35 μl of non washed red blood cell pellet (from 80 to 90%) near each drop of reagent.
3. For each test, mix reagent and red blood cells with a glass rod over a circle area of 2-3 cm diameter.
4. Repeat steps 1. to 3. with RH Control M when its use is required.
5. Change the glass rod for each reaction.
6. Gently rotate the plate and observe for agglutination at 3 minutes.

**B - Tube method (immediate spin)**

1. Prepare a 3 to 5% suspension in isotonic saline of the red blood cells to be tested.
2. Into a labelled disposable tube, distribute 50 μl of the reagent.
3. Add 50 μl of red blood cell suspension to be tested.
4. Repeat steps 2. and 3. with RH Control M when its use is required.
5. Mix thoroughly by gently shaking the tube.
6. Centrifuge for 1 minute at 450 g.
7. Gently resuspend each cell button from the bottom of the tube and observe macroscopically for agglutination.

**C - Microplate method (+15°C - +25°C)**

a) **Preparation of the reagent**

1. Add 1.25 mL of TransClone Anti-RH1 (D) FAST M in a 5 mL vial of Microdil.
2. Mix.
3. The reagent, diluted like this, is ready for use for microplate method and can be stored for 7 days between +2°C and +8°C.
b) Technique
1. Into a labelled disposable tube or in a microplate designed for this purpose, prepare a 2 % suspension in Microbrom of the red blood cells to be tested.
2. Incubate between 5 and 20 minutes at room temperature (+15°C - +25°C).
3. For each sample, distribute, into a U shaped microplate, 25 μl of reagent as prepared in C-a) into one well and 25 μl of RH Control M into an other well.
4. Resuspend the red blood cells.
5. Add 25 μl of red blood cell suspension to be tested into the corresponding wells.
6. Homogenize the reaction mixture using the microplate shaker.
7. Centrifuge 1 minute at 250 g.
8. Shake the microplates one at a time so as to dislodge the cell button and to allow for a good resuspension of the negative controls. The agitation parameters must be established according to the microplate shaker used.
9. Incubate 2 minutes at room temperature and read.

VII - RESULTS AND INTERPRETATION
- Agglutination of the red blood cells with the reagent corresponds to a positive result and indicates the presence of the RH1 (D) antigen.
- No agglutination of the red blood cells corresponds to a negative result and indicates that the RH1 (D) antigen has not been detected.
- The results are validated only if the positive and negative controls give the expected results and if the RH Control M shows a negative result when it is used.

VIII - PERFORMANCE
The performance of TransClone Anti-RH1 (D) FAST M reagent has been evaluated on a panel of 2078 samples (609 donors, 1412 patients and 57 new born samples) completed with a panel of 52 special samples (weak or variant Ag). The 2078 samples including 82% positive samples for RH1 (D) Ag have given compliant results of sensitivity and specificity with the expected ones. 95% of the RHW1 (weak D) Ag tested have been detected in tube technique. In spite of its performance, TransClone Anti-RH1 (D) FAST M reagent does not allow the detection of all the RHW1 (weak D) antigens, neither the detection of the phenotype RH1 partial category VI (DVI). If the detection of all the RHW1 (weak D) and RH1 (D) variant antigens is required, the samples found negative must be confirmed by the research of RHW1 (weak D) antigen using the indirect antiglobulin technique (for example Scangel Monoclonal Anti-RH1 (D)/RHW1 reagent together with the Scangel COOMBS Anti-IgG,-C₃d card).
TransClone Anti-RH1 (D) FAST M reagent showed good reproducibility in both intra and inter tests.
LIMITS
Abnormal results may be caused by:
- bacterial or chemical contamination of the samples, reagents or equipment.
- patient medication or disease yielding a cross-reaction.
- use of a sample dilution medium other than that recommended (microplate method only).
- a red blood cell preparation different to that recommended.
- an insufficient agitation involving an incomplete resuspension of the red blood cells.
- a too strong agitation breaking the agglutinates.
- use of other procedure than the one described above.

IX - LITERATURE
• CE marking (European directive 98/79/CE on in vitro diagnostic medical devices)
• Marquage CE (Directive européenne 98/79/CE relative aux dispositifs médicaux de diagnostic in vitro)
• Marco CE (Directiva europea 98/79/CE sobre productos sanitarios para diagnóstico in vitro)
• EG Markierung (Europäische Richtlinie 98/79/EG über In-vitro-Diagnostika)
• Marchiatura CE (Direttiva europea 98/79/CE relativa ai dispositivi medico-diagnostici in vitro)
• Marcação CE (Directiva europeia 98/79/CE relativa aos dispositivos médicos de diagnóstico in vitro)
• CE-märkning (Europap-direktiv 98/79/EG om medicintechniska produkter lör in vitro-diagnostik)
• CE-mærkning (Europa-direktiv 98/79/EF om medicinsk udstyr til in vitro-diagnostik)

**IVD**
- For in vitro diagnostic use
- Pour diagnostic in vitro
- Para diagnóstico in vitro
- In vitro-Diagnostikum
- Per uso diagnostico in vitro
- Para uso em diagnóstico in vitro
- In vitro-diagnostik
- In vitro-diagnose

**EC REP**
- Authorised Representative
- Représentant agréé
- Representante autorizado
- Bevollmächtiger
- Distributore autorizzato
- Representante Autorizado
- Auktoriserad representant
- Autoriseret repræsentant

**LOT**
- Batch code
- Code du lot
- Código de lote
- Chargen-Bezeichnung
- Codice del lotto
- Código do lote
- Batch nr.
- Batchnummer

**REF**
- Catalogue number
- Référence catalogue
- Número de catálogo
- Bestellnummer
- Numero di catalogo
- Número de catálogo
- Katalognummer
- Katalognummer

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