BIO-RAD

TransClone® Anti-ABO1 (A) 26A2

86328 (1 x 10 ml) 86320 (10 x 10 ml)
86982 (100 x 10 ml) 86422 (1l)
86934 (10 l)

TransClone® Anti-ABO2 (B) X9

86470 (1 x 10 ml) 86471 (10 x 10 ml)
86472(100 x 10 ml) 86469 (10l)

TransClone® Anti-ABO3 (AB)

86362 (1 x 10 ml) 86363 (10 x 10 ml)
86984(100 x 10 ml) 86986 (1 l)
86943 (10 l)

MURINE MONOCLONAL ANTIBODIES
ABO1 (A) Ag determination
ABO2 (B) Ag determination
ABO3 (AB) Ag determination
SLIDE (+15°C - +25°C) **TUBE** (IMMEDIATE SPIN)
AND MICROPLATE (+15°C - +25°C)

IVD

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

These reagents (serum-test) are strictly reserved for professional and *in vitro* diagnostic use.

Designed for the determination of ABO1 (A) antigen or for the determination of ABO2 (B) antigen or for the determination of ABO3 (AB) antigen, the test is based on the principle of agglutination.

Red blood cells possessing the tested antigen will agglutinate in the presence of reagent whereas red blood cells lacking the 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 REAGENTS

These reagents are supplied in liquid form, ready for use, provided with droppers (one drop = 50 µl).

They contain monoclonal antibodies issued from clones produced through cell fusion of immunized mice lymphocytes and murine myeloma cells. The supernatants are produced by the following clones :

- TransClone Anti-ABO1 (A) 26A2 : 26A2
- TransClone Anti-ABO2 (B) X9 : X9
- TransClone Anti-ABO3 (AB) : AB5-63A5A2/26A2/95.3

These reagents contain 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 each box label.

III - STORAGE – SHELF LIFE

The expiry date and storage conditions are stated on each 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 reagents are 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.
- Use a different tip for each reagent.
- It is essential to take precautions in order not to provoke contamination, particularly during the distribution steps.
- 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°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 datasheets are 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-ABO1 (A) 26A2 or TransClone Anti-ABO2 (B) X9 or TransClone Anti-ABO3 (AB)

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 studied 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. Into a labelled disposable tube, prepare a 10 to 50% suspension in isotonic saline of the red blood cells to be tested.
2. On a slide, at room temperature (+15°C - +25°C), deposit 50 µl of the reagent.
3. Add 50 µl of red blood cell suspension near the drop of reagent.
4. For each test, mix reagent and red blood cells with a glass rod over a circle area of 2-3 cm diameter.
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. Mix thoroughly by gently shaking the tube.
5. Centrifuge for 1 minute at 450 g.
6. 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 reagents

- Add :
  - 1.25 ml of TransClone Anti-ABO1 (A) 26A2 to a 5 ml vial of Microdil
  - 0.75 ml of TransClone Anti-ABO2 (B) X9 to a 5 ml vial of Microdil
  - 1.25 ml of TransClone Anti-ABO3 (AB) to a 5 ml vial of Microdil
- Mix.

- The reagents, diluted like this, are ready for use for microplate method and can be stored for 7 days between +2°C and +8°C.

b) Method

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 red blood cells in the presence of reagent corresponds to a positive result and indicates the presence of the corresponding antierythrocytic antigen.
- No agglutination of the red blood cells corresponds to a negative result and indicates that the corresponding 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.**

Complete ABO grouping requires 2 complementary tests : the forward test conducted with anti-ABO1 (A), anti-ABO2 (B) reagents and if necessary anti-ABO3 (AB) reagent, and the reverse test conducted with the A1, B reagent red blood cells and if necessary A2 and O reagent red blood cells. The profiles of the expected results with anti-ABO1 (A), anti-ABO2 (B), anti-ABO3 (AB) reagents and A1, A2, B, O reagent red blood cells and their interpretation are presented in the following table :

GROUPS	ABO FORWARD TEST : REAGENTS			ABO REVERSE TEST : REAGENT RED BLOOD CELLS			
	Anti-ABO1 (A)	Anti-ABO2 (B)	Anti-ABO3 (AB)	A1	A2	B	O
A	+	-	+	-	-	+	-
B	-	+	+	+	+	-	-
AB	+	+	+	-	-	-	-
O	-	-	-	+	+	+	-

The forward and the reverse test results must concord. Any discrepancy between those two tests must be resolved before any ABO result can be given. Whenever the forward and reverse test results conflict, complementary tests with appropriate controls are to be conducted.

VIII - PERFORMANCE

a) Specific performance of TransClone Anti-ABO1 (A) reagent

The performance of TransClone Anti-ABO1 (A) 26A2 reagent has been evaluated on a panel of 5763 samples (5100 donors, 625 patients and 38 new born samples) completed with a panel of weak antigens.

Samples (including 47% of positive Ag ABO1 (A)) have given compliant results of sensitivity and specificity with the expected ones.

TransClone Anti-ABO1 (A) 26A2 reagent showed good reproducibility in both intra and inter tests.

b) Specific performance of TransClone Anti-ABO2 (B) reagent

The performance of TransClone Anti-ABO2 (B) X9 reagent has been evaluated on a panel of 1356 samples (457 donors, 879 patients and 20 new born samples) completed with a panel of weak antigens.

The tested acquired B antigens were not detected by the TransClone Anti-ABO2 (B) X9 reagent.

Samples (including 15% of positive Ag ABO2 (B)) have given compliant results of sensitivity and specificity with the expected ones.

TransClone Anti-ABO2 (B) X9 reagent showed good reproducibility in both intra and inter tests.

c) Specific performance of TransClone Anti-ABO3 (AB) reagent

The performance of TransClone Anti-ABO3 (AB) reagent has been evaluated on a panel of 6492 samples (4290 donors, 2111 patients and 91 new born samples) completed with a panel of weak antigens.

Samples (including 59% of positive Ag ABO3 (AB)) have given compliant results of sensitivity and specificity with the expected ones.

TransClone Anti-ABO3 (AB) 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

1. Arrêté du 8 février 1984. *Journal Officiel N.C.* du 17 mars 1984 fixant les caractéristiques des réactifs utilisés en immunohématologie érythrocytaire.
2. Technical Manual of the American Association of Blood Banks. 10th edition 1990.
3. CH. Salmon, J.P. Cartron, PH. Rouget et collaborateurs - Les groupes sanguins chez l'homme. Masson 1991.
4. Issitt P.D.: Applied blood group serology. 4th ed. Miami : *Montgomery Scientific Publications,* 1998.

- 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*)
- Marcodo 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 (Europa direktiv 98/79/EG om medicintekniska produkter lör *in vitro*-diagnostik)
- CE-mærkningen (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

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

- Manufacturer
- Fabricant
- Fabricante
- Hersteller
- Produttore
- Fabricante
- Tillverkad av
- Fremstillet af

EC REP
- Authorised Representative
- Représentant agréé
- Representante autorizado
- Bevollmächtigter
- 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.
- Batchkoden

- Expiry date YYYY/MM/DD
- Date de péremption AAAA/MM/JJ
- Estable hasta AAAA/MM/DD
- Vervvendbar bis JJJJ/MM/TT
- Da utilizzare prima del AAAA/MM/GG
- Data de expiração AAAA/MM/DD
- Utgångsdatum År/Månad/Dag
- Anvendes før ÅÅÅÅ/MM/DD

- Storage temperature limitation
- Limites de températures de stockage
- Temperatura limite
- Lagerungstemperatur
- Limiti di temperatura di conservazione
- Limites de temperatura de armazenamento
- Temperaturbegränsning
- Temperaturbegrænsning

- Consult Instruction for use
- Consulter le mode d'emploi
- Consulte la instrucción para el uso
- Siehe Gebruchsanweisung
- Consultare le istruzioni per uso
- Consulte o folheto Informativo
- Se instruktionsanvisning vid användning
- Se instruktion før brug

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