Biotestcell-I8 / Biotestcell-I11

Reagent Red Blood Cells for antibody identification

REF	816020	VOL 8x 4 ml	Biotestcell-I8
REF	816021	VOL 11 x 4 ml	Biotestcell-I11

INTENDED USE

Biotestcell-I8 are Reagent Red Blood Cells intended to be used for the qualitative identification of unexpected human red cell antibodies in donor and patient (recipient) plasma/serum in the manual tube method. Whole blood collected in EDTA, citrate or without anticoagulant may be used.
For in vitro diagnostic use, by trained laboratory personnel.

Biotestcell-I11 are Reagent Red Blood Cells intended to be used for the qualitative identification of unexpected human red cell antibodies in donor and patient (recipient) plasma/serum in the manual tube method. Whole blood collected in EDTA, citrate or without anticoagulant may be used.
For in vitro diagnostic use, by trained laboratory personnel.

TEST PRINCIPLE

The test principle is haemagglutination.
When corresponding antibodies bind to the antigens of Reagent Red Blood Cells, agglutinates are formed directly or after addition of anti-human globulin, resulting in a positive reaction after following the test procedure [1,2,3].

REAGENT

The Biotestcell-I8 / Biotestcell-I11 Reagent Red Blood Cells are from single human donors in separate vials, suspended at 3% in modified Alsevers solution and are ready to use reagents.

Biotestcell-I8 / Biotestcell-I11* contain the following antigens: D, C, C^w, E, c, e, K, k, Kp^{a*}, Kp^b, Fy^a, Fy^b, Lu^a, Lu^b, Jk^a, Jk^b, Js^{a*}, Js^b, M, N, S, s, Le^a, Le^b, P_1, Xg^a, Co^a, Di^{a*}. For the exact antigen content of each production lot, please refer to the enclosed antigen table.
*Only applicable for Biotestcell-I11.

PRES Neomycin Sulfate, Chloramphenicol, Amphotericin B

STORAGE CONDITIONS AND SHELF LIFE

The product has to be stored at 2 – 8 °C. After opening the product can be used until the expiry date (see label), if stored at 2 – 8 °C.

WARNING AND PRECAUTIONS

- Do not use damaged vials.
- Do not mix vials of different lots as reactions patterns for result interpretation vary.
- The Reagent Red Blood Cells should not be used if the cells are darkly discoloured, spontaneously agglutinated or show considerable haemolysis or turbidity.
- These instructions must be strictly followed by a trained healthcare professional to achieve accurate results. Each deviation from these instructions is the sole responsibility of the user.
- These devices should be handled only by qualified personnel trained in laboratory procedures and familiar with the potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately in accordance with Good Laboratory Practices.
- The primary packaging of this product contains natural rubber latex which may cause allergic reactions.
- Source material from which this product was derived was found negative for anti-HIV-1/-2, anti-HCV, HBsAg and anti-Lues. Nevertheless, all test reagents of biological origin must be regarded as potentially infectious and appropriate safety precautions are recommended.
- Test material must be discarded as hazardous material. Waste management information can be found in the safety data sheet (www.bio-rad.com)
- Consult **downloads.bio-rad.com** to download the valid version of the instructions for use.

SPECIMEN COLLECTION

Blood samples should be drawn following general blood sampling guidelines.
EDTA or citrate anticoagulated samples and samples without anticoagulant may be used for testing. Prior to testing, samples must be centrifuged according to local laboratory practices to obtain a distinct separation between cells and plasma/serum. Blood samples should be tested as soon as possible post collection. If testing is delayed, blood samples shall be stored at 2 - 8 °C and tested within 10 days post collection.

MATERIALS

Material Provided

- Biotestcell-I8
- Biotestcell-I11

Material Required but not Supplied

- Glass tubes
- Pipettes (drop volume 50µl)
- Isotonic saline
- Laboratory centrifuge
- Water bath, ID-Incubator L or equivalent
- Anti-Human Globulin REF 804020/Anti-Human Globulin Color REF 804120, 804115 and 804130
- MLB 2 REF 805205, 805200, 12014332
- Rinderalbumin 22 % REF 805060
- Rinderalbumin 30 % REF 805090
- Bromelin REF 805012
- Papain REF 805030
- Coombscell-E REF 816030

TEST PROCEDURE

Allow reagents and samples to reach room temperature (18-25°C) before use. Gently resuspend the Reagent Red Blood Cells by inverting the vial several times before use.

3-Phase Tube Test for Antibody Detection

For each of the Reagent Red Blood Cells of the Biotestcell-I8 / Biotestcell-I11 proceed as follows:

Phase 1: Immediate Spin

1. Place 2 drops (100 µl) of plasma/serum into the appropriately marked tubes, add 1 drop (50 µl) of Reagent Red Blood Cells and mix well.
2. Centrifuge for 20 seconds at 800 - 1000 x g
3. Gently dislodge cell button and observe for agglutination.
4. Record results.

Phase 2: Spin Test after Incubation

5. Incubate 30 - 60 minutes at 37 °C.
6. Centrifuge for 20 seconds at 800 - 1000 x g.
7. Gently dislodge the cell button and observe for agglutination.
8. Record results.

Phase 3: Indirect Antiglobulin-Test (IAT)

9. Wash the Reagent Red Blood Cells 3 times with isotonic saline. Decant supernatant completely.
10. Add 2 drops (100 µl) of anti-human globulin to the corresponding packed Reagent Red Blood Cells and mix.
11. Centrifuge for 20 seconds at 800 - 1000 x g.
12. Gently dislodge the cell button and observe for agglutination.
13. Record results.

To control all negative results, add Coombscell-E (Reagent Red Blood Cells sensitized with IgG antibody) as positive control, please refer to the respective instruction for use.

If enzymes or supplements (Bromelin, Papain, Rinderalbumin 22%, Rinderalbumin 30 % or MLB 2) is used, please refer to the respective instructions for use.

STABILITY OF THE REACTION

After test procedure, the results should be read immediately to avoid false results.

INTERPRETATION OF RESULTS

Agglutination of the Reagent Red Blood Cells is a positive result. A positive reaction with Biotestcell-I8 / Biotestcell-I11 indicates the presence of antibodies against the respective antigen (see enclosed antigen table). For verification an additional antigen test identification should be performed.

No agglutination is a negative result. Negative results in the 3-phase-test and a positive reaction with the Coombscell-E, indicates that the plasma/serum contains no detectable antibodies against one of the listed antigens (see enclosed antigen table).

Reaction strength	Agglutination	Result Interpretation
4+	One solid agglutinate	Positive
3+	Several large agglutinates, clear background	Positive
2+	Medium-size agglutinates, clear background	Positive
1+	Few small agglutinates, turbid background	Positive
+/-	Barely visible agglutinates, turbid background	Positive
-	No agglutination, an even red blood cell suspension	Negative

For technical support, visit the contact us section, at www.bio-rad.com website. Then select a location and select “Clinical Diagnostics”.

QUALITY CONTROL

The reactivity of all blood typing reagents should be confirmed on each day of use. Known positive and negative samples should be included in accordance with local guidelines.

LIMITATIONS

- Neonatal specimens were not tested for these reagents.
- Low frequency antigens may not always be present on the Reagent Red Blood cells. Therefore, negative reactions with the detection Reagent Red Blood Cells do not always indicate the absence of unexpected antibodies.
- Because some antibodies show a dosage effect, the antigen density on the Reagent Red Blood Cells needs to be considered when evaluating the test results (homozygous or heterozygous hereditary disposition). A heterozygous expression of the antigen may result in non-detection of weak antibodies depending on the used test method.
- In very rare cases HLA Class I Antigens or low frequent antigens within the product may lead to positive reactions.
- Agglutinins specific for papain-treated RBCs can be found in serum or plasma and cause agglutination of reagent red cells in IAT techniques.
- False negative reactions may be obtained if the sample contains antibodies present in concentrations too low to be detected by the test method employed. No test method is capable of detecting all red blood cell antibodies.
- Insufficient or inappropriate washing can lead to false reactions. Small amounts of residual plasma/serum can neutralize the Anti-Human Globulin Anti-IgG.

Bio-Rad Medical Diagnostics GmbH
Industriestr. 1, 63303 Dreieich, Germany
www.bio-rad.com

CE 0123

- Blood samples from patients who have received plasma expanders of high molecular weight may give false positive results.
- Further conditions that may cause false results are:
  - Cold antibodies
  - Microbial contamination of sample or reagent
  - Auto-antibodies
  - High Titer Low Avidity (HTLA) antibodies
  - Panagglutinins
  - Patient's medication (e.g. antibiotics or monoclonal antibodies)
  - Patient's disease.
  - Contamination of cord blood samples with Wharton's jelly
- The reactivity of the product may decrease during shelf-life. The rate of decrease in reactivity is partially dependent on individual donor characteristics that are neither controlled nor predicted by the manufacturer.
- Haemolysed, lipemic or icteric blood samples above the tested values may interfere with the results.
- Fibrin, clots, particulates or other artifacts may cause an anomalous result.
- Excessive agitation may disrupt weak agglutination and produce false negative results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Clinical Performance

Clinical performances of antibody identification have been evaluated on samples from fresh and frozen donors and patients in tube method. Positive samples targeted the main blood group systems.

Agreements on antibody specificity identification with reference method bearing CE marking was calculated as follows:

- At least 2 positive reactions with antigen positive Reagent Red Blood Cell or all positive antigen Reagent Red Blood Cell reacts positively.
- At least 2 negative reactions with antigen negative Reagent Red Blood Cell

RRBCs	Method	Number of samples tested	Overall % Agreement [95% Exact Lower CI]
Biotestcell-I8	3-phase	59	98.31% 58/59 [92.21%]
Biotestcell-I8	Addition of MLB2	59	98.31% 58/59 [92.21%]
Biotestcell-I11	3-phase	59	96.61% 57/59 [89.71%]
Biotestcell-I11	Addition of MLB2	59	98.31% 58/59 [92.21%]

Clinical performances were also evaluated by considering each Reagent Red Blood Cell reaction separately. Thus, performance regarding the identification of the antibody, which is country specific (rule-in/rule-out antibodies) and dependent on number of Reagent Red Blood Cells tested, was not considered. For this analysis, the reaction was expected positive when the antigen was present on the Reagent Red Blood Cells (as described in the provided antigen table) and the corresponding antibody specificity was present in the sample. The reaction was expected negative when the antigen was absent on the Reagent Red Blood Cells.

Method	Number of samples tested	Sensitivity* [95% Exact Lower CI]	Specificity [95% Exact Lower CI]
3-phase	60	99.18% [97.44%]	99.76% [98.86%]
Addition of MLB2	60	99.18% [97.44%]	100.00% [99.28%]
Bromelin	20	100% [71.69%]	66.67% [54.29%]
1-step Papain	20	66.67% [34.49%]	94.12% [85.49%]
2-step Papain	20	100% [71.69%]	98.04% [91.03%]

* Default of performance in sensitivity includes negative reactions on single dose Reagent Red Blood Cells, which may be expected for antibody showing dosage effect.

Analytical Specificity

Potential interfering substances (bilirubin, total proteins, triglycerides and haemoglobin) at concentration shown in the table below, were tested in tube and have no impact on Biotestcell-I8 and Biotestcell-I11 performances.

Interfering Substance	Concentration tested without enhancers or enzymes	Concentration tested with enhancers or enzymes
Human Haemoglobin	1000 mg/dL	400 mg/dL
Bilirubin unconjugated	40 mg/dL	29 mg/dL
Triglyceride (Intralipid)	1500 mg/dL	500 mg/dL
Human Albumin (Total Proteins)	15 g/dL	15 g/dL

Precision Performance (Repeatability/Reproducibility)

Performances related to repeatability and reproducibility inter-instruments, inter-operators, inter-lots, inter-days and inter-runs have been evaluated on 4 samples (2 positive and 2 negative samples) for at least one of the reagents red blood cell of the Biotestcell-P3.

Repeatability was evaluated according to the following schema: 1 lot of reagent, 1 operator, 1 instrument, 3 replicates, 5 non-consecutive days and 2 runs/day.

Lot to lot reproducibility was evaluated according to the following schema: 3 lots of reagent, 1 operator, 1 instrument, 3 replicates, 5 non-consecutive days and 2 runs/day.

Reproducibility was evaluated according to the following schema: 1 lot of reagent, 3 operators, 3 instruments, 3 replicates, 5 non-consecutive days and 2 runs/day.

Method	Repeatability		Reproducibility	
	Positive % Agreement [95% Exact Lower CI]	Negative % Agreement [95% Exact Lower CI]	Positive % Agreement [95% Exact Lower CI]	Negative % Agreement [95% Exact Lower CI]
3-phase	100% 60/60 [95.13%]	100% 60/60 [95.13%]	100% 180/180 [98.35%]	100% 180/180 [98.35%]
Addition of enhancer*	100% 60/60 [95.13%]	100% 60/60 [95.13%]	100% 180/180 [98.35%]	100% 180/180 [98.35%]
Bromelin	100% 60/60 [95.13%]	100% 60/60 [95.13%]	100% 180/180 [98.35%]	100% 180/180 [98.35%]
1-step Papain	100% 60/60 [95.13%]	100% 60/60 [95.13%]	100% 180/180 [98.35%]	100% 180/180 [98.35%]
2-step Papain	100% 60/60 [95.13%]	100% 60/60 [95.13%]	100% 180/180 [98.35%]	100% 180/180 [98.35%]

*Enhancers tested are MLB2, Rinderalbumin 22% and 30%. Consistent performances obtained for all enhancers.

NOTE

- For a patient/user/third party in the European Union and in countries with identical regulatory regime requirements (Regulation 2017/746/EU on In vitro Diagnostic Medical Devices); if during the use of this device or as a result of this use, a serious incident occurs, please report it to Bio-Rad Medical Diagnostics GmbH and to your national Competent Authority.
- For the European Union (Regulation 2017/746/EU), the Summary of Safety and Performance of this device is available via EUDAMED public access https://ec.europa.eu/tools/eudamed.

GLOSSARY OF SYMBOLS

The following symbols **may** have been used in the labelling of this product.

Symbol	Explanation	Symbol	Explanation
LOT	Batch Code or Lot Number	IVD	*In vitro* diagnostic medical device
⚠	Consult the warnings and precautions		Consult electronic instructions for use
	Manufacturer	VOL	Volume
PRES	Preservative	REF	Catalogue number
	Temperature limitation		Keep away from sunlight
LATEX	Contains or presence of natural rubber latex		Use by or expiration date (YYYY-MM-DD)
CE 0123	Conformity with European Regulation (EU) 2017/746 certified by notified body number 0123		

REFERENCES

1. Daniels G. Human Blood Groups. 3rd ed. Oxford: Wiley-Blackwell, 2013.
2. Marion E. Reid, Christine Lomas-Francis and Martin L. Olsson, THE BLOOD GROUP ANTIGEN FactsBook. 3rd ed. Oxford: Elsevier; 2012
3. Peter D. Issit, David J. Anstee, Applied Blood Group Serology Fourth Edition, Montgomery Scientific Publications Durham, NorthCarolina, USA, 1998

Key: Underline = Addition or significant change ◄= Deletion of text