REAGENT RED BLOOD CELLS

A1 Cells / B Cells

REF 17318

Manufacured by:
DIAOAST
251, Avenue Eugène Armand
EureSCAN Pac
93820 LOOS - FRANCE

Formulated for Use in Automated Systems
Beckman Coulter PK Systems
141EN012: September 2020

For in vitro diagnostic use
No U.S. standard of potency
Do not use if hemolyzed
Do not freeze
Preservatives: Neomycin Sulfate, Gentamycin Sulfate, Thiamphenicol, Sulfathiazole

I. INTENDED USE

The PK SYSTEM REAGENT RED BLOOD CELLS (A1 and B) are intended for the determination of the reverse or plasma group on the BECKMAN Coulter PK7300 and PK7400 Automated Microplate System(s).

II. SUMMARY AND EXPLANATION

The determination of an ABO blood group is defined by demonstrating the presence or absence of antigens A and/or B and anti-A and anti-B antibodies in the serum. It is therefore appropriate to identify the red blood cells antigens using known A, anti-A, anti-B and anti-A,B reagents (red blood cells or forward group), then to confirm the preceding result by verifying the presence of the corresponding antibodies in the plasma by using known red blood cells A and B (plasma or reverse group). Discrepancies should be resolved before final interpretation of the ABO group.

III. THE PRINCIPLE ANTIGENS AND ANTIBODIES OF THE ABO SYSTEM

<table>
<thead>
<tr>
<th>ABO Blood Group</th>
<th>Antigen present on the red blood cells</th>
<th>Antibodies regularly present in the serum/plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>neither A nor B</td>
<td>anti-A and anti-B</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>A and anti-B</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>anti-A, anti-B</td>
</tr>
<tr>
<td>AB</td>
<td>A and B</td>
<td>none</td>
</tr>
</tbody>
</table>

IV. PRINCIPLE OF PROCEDURE

The test is based on hemagglutination principles. Reagent red blood cells with specific antigens agglutinate in the presence of corresponding antibodies contained in donor plasma. The absence of agglutination indicates the absence or weakened expression of the specific antibody in the donor plasma. The PK7300 and PK7400 analyzers will read the settling patterns of the red blood cells in each well of the microplate and make a determination based on the threshold settings chosen for each reagent. For complete details on the setup and operation of the BECKMAN COULTER PK7300 and PK7400 refer to the respective User's Guide and Instructions for Use.

V. REAGENTS

The PK SYSTEM REAGENT RED BLOOD CELLS is a suspension of pooled red blood cells. Each bottle contains either group A or B red blood cells. The NTD rate may be higher due to the use of 9th-positive blood in the group B Reagent Red Blood Cells. The red blood cells are resuspended to a concentration of 2% (+/- 0.5) in Abee's solution containing neomycin sulfate, gentamycin sulfate, thiamphenicol and sulfathiazole as preservatives.

VI. Warnings and Precautions

1. PRECAUTIONS: PK SYSTEM REAGENT RED BLOOD CELLS ARE OF HUMAN ORIGIN. ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIAL FROM WHICH THESE PRODUCTS WERE DERIVED WAS FOUND NEGATIVE WHEN TESTED IN ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS.

2. Avoid cross-contamination of reagents or specimens. Do not pipette any reagents by mouth. All blood products should be treated as potentially infectious.

3. The microplates must be clean and dry before use. Improper cleaning of the microplates can adversely affect the test result by causing a false-negative or false-positive reaction. The suggested cleaning procedures for the PK microplates can be found in the PK7300 User's Guide and PK7400 Instructions for Use.

4. Visible signs of microbial growth or gross hemolysis in any reagent may indicate degradation and warrant discontinuance of use.

5. Carryover between specimens is a potential source of interference.

6. Microbial contamination of the specimen may produce effects that cannot be predicted.

7. Positive and negative control material should be handled in the same fashion as donor samples.

8. Incorrect sampling of the sample, diluent or reagent could result in erroneous test results.

9. Failure to follow directions contained in the instructions for use may result in erroneous results.

10. The use of calibrated or verified equipment is required.

11. Phosphate Buffered Saline (PBS) should NOT be used in the test system.

12. Effort should be made to prevent contamination and evaporation during use of the product.

13. Do not pool or transfer reagents in or between vials in any manner. Do not transfer reagent from a new vial to an open vial. Do not transfer reagent from an open vial to any other container.

14. Reagents should not be used past the expiration date.

15. Agglutination may be weaker with older specimen samples than with those from freshly drawn blood and may result in a higher than determined (NTD) rate.


VII. STORAGE

1. Store reagents at +2°C to +8°C when not in use. Store vials in an upright position when not in use. Do not freeze.

2. Do not use reagents beyond the expiration date.

3. Reagents left on board on the BECKMAN COULTER PK Systems for 12 hours or more should be discarded.

VIII. SPECIMEN COLLECTION AND PREPARATION

1. No special preparation of the donor is required prior to specimen collection. Blood samples must be collected in EDTA anticoagulant in either glass or plastic tubes. Clotted samples should not be used when red cell testing is being carried out.

2. Specimens from donors with protein abnormalities may give erroneous results on the PK7300 and PK7400 Lipemic, icteric or hemolyzed samples may produce erroneous results in plasma ABO testing (reverse ABO grouping). Anticoagulated samples containing clots may also give erroneous results in ABO cell testing.
3. If testing must be postponed for longer than 24 hours from collection, the specimen must be stored at +2°C to +8°C. Return to room temperature (+15°C to +30°C) prior to analysis. Testing should be carried out within five (5) days of collection (see Warnings and Precautions #15).

4. Bacterial contamination of the specimen may cause erroneous test results.

5. Proper centrifugation of the samples is necessary to achieve optimum performance of the PK7300 and
PK7400. False-positive results may be observed in tests involving plasma from the sample if particulate
matter is not removed during centrifugation. In order to minimize remixing of plasma with cells in the sample,
avoid or minimize applying the brake at the end of centrifugation.

- To prepare samples for analysis:
  - Prepare cells for centrifugation by inverting the sample.
  - Thoroughly mix and centrifuge samples within 10 hours of analysis on the PK7300 and PK7400.
  - Centrifuge samples for a minimum of 10 minutes at 1500 x g.

Note: Centrifugation speed and time may need to be varied depending on sample age, time between
centrifugation and analysis, and storage temperature. For further details refer to the PK7300 User’s Guide
and PK7400 instructions for use.

IX. MATERIALS

MATERIALS PROVIDED
- PK SYSTEM REAGENT RED BLOOD CELLS, A1 and B red blood cells for reverse grouping

MATERIALS REQUIRED BUT NOT PROVIDED
- BECKMAN COULTER PK7300 and/or PK7400 Automated Microplate System(s)
- BECKMAN COULTER terraced microplates
- Centrifuge
- Control samples (positive and negative)
- Physiological (0.85-0.9%) saline for plasma sample diluent.

Note: Phosphates Buffered Saline (PBS) is not suitable.

The PK7300 and PK7400 are programmable analyzers, the operation of which is controlled by user defined
software settings. A list of recommended parameters and threshold settings for ABO grouping on the
PK7300 and PK7400 is shown below. Good laboratory practice dictates that each laboratory validates the
operating parameters. For further information, please consult Section D of the PK7300 User’s Guide and/or
Chapter 3 of the PK7400 Instructions for Use.

X. DIRECTIONS FOR USE

PK7300 RECOMMENDED PARAMETERS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sam-Je Volume</td>
<td>120 L</td>
</tr>
<tr>
<td>Diluent Volume</td>
<td>132 L</td>
</tr>
<tr>
<td>Sam-Je/Diluent Ratio</td>
<td>2:1</td>
</tr>
<tr>
<td>Diluted Sam-Je Volume</td>
<td>25 L</td>
</tr>
<tr>
<td>Reagent Volume</td>
<td>25 L for both A1 Cells and B Cells</td>
</tr>
<tr>
<td>Channel Name</td>
<td>Variable</td>
</tr>
<tr>
<td>Channel Design</td>
<td>1-12</td>
</tr>
<tr>
<td>Decision Lo-J0 Rules</td>
<td>+/-</td>
</tr>
<tr>
<td>Temperature Setting</td>
<td>28°C</td>
</tr>
<tr>
<td>Incubation Time</td>
<td>60 minutes</td>
</tr>
<tr>
<td>Microplate Well</td>
<td>16 μm</td>
</tr>
</tbody>
</table>

PK7400 RECOMMENDED PARAMETERS

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<tr>
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<tr>
<td>Microplate Well</td>
<td>16 μm</td>
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</tbody>
</table>

PK7300 OPERATING INSTRUCTIONS

Proceed with sample analysis as outlined in Basic Operations, Chapter C of the BECKMAN COULTER PK7300
User’s Guide.

PK7400 OPERATING INSTRUCTIONS

Proceed with sample analysis as outlined in Basic Operations, Chapter 2 of the BECKMAN COULTER PK7400
Instructions for Use.

XI. QUALITY CONTROL

A series of quality control samples should be run at the beginning and end of each test run. A “test run” is defined
as an uninterrupted analysis of test samples not to exceed 500 samples on a single analyzer. Interruptions in
processing could include but are not limited to:
- changes in reagent lot number
- delays caused by electronic or mechanical malfunction
- addition of reagent or diluant

For the results of a sample test run to be considered valid, a positive and negative control at the beginning and
end of each run should provide the expected results.

Quality control samples should be tested in the same manner as all other samples. The control samples should
produce positive (+) reactions and the negative controls should produce negative (-) reactions with the appropriate
reagent. If the expected results are not obtained with an individual control sample, the suspect quality control sample
should be retested for both adequate quantity and compliance with the sample requirements. Failure of controls to
perform as expected may indicate contamination or deterioration of one or more of the reagents comprising the
system. When the expected results with control materials are not obtained, contact BECKMAN COULTER
Technical Service at 800-447-5652. Please refer to the PK7300 User’s Guide and PK7400
Instructions for use for additional information concerning the use of control samples.

XII. INTERPRETATION

The PK7300 and PK7400 will read the settling patterns of the red blood cells in each well based on the threshold
settings shown. Refer to Section G in the BECKMAN COULTER PK7300 User’s Guide and
Appendix A of the BECKMAN COULTER PK7400 Instructions for use for complete details of the manner in which the
analyzer interprets reactions.

Results should be verified by visual review of the reaction patterns in the microplate wells against the analyzer
printout. The PK7300 and PK7400 stores an actual image of the microplate and visual review may be performed
at the operator’s convenience. All plates should be visually reviewed. Visually, a positive test is a homogeneous

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layer of cells. Visually a negative test would result in a compact dense button surrounded by a clear zone. Additional testing must be performed on any sample for which visual and analyzer interpretations do not agree. The sequence of reactions for ABO, are compared to user-defined logic for ABO blood group determination.

XIII. INTERPRETATION OF RESULTS

A person’s ABO blood group is determined by testing the red blood cells with Anti-A and Anti-B. “Agglutination” of the test cells indicates the presence of the relevant antigen, while no agglutination indicates its absence. A positive reaction in the test with Anti-A indicates the presence of the A antigen in the red blood cells of the person, whereas a negative reaction may or may not react with Anti-A, depending on the antigen that is expressed on the particular cells. Most examples of A antigens are a subgroup of (such as A1). Red blood cells of the A antigens may not react with Anti-A, depending on the strength to which the antigen is expressed on the particular cells. Most examples of A antigens are a subgroup of (such as A1).

Confirmation of the test results, is provided by testing the serum or plasma of the blood under investigation with group A and group B red blood cells, and by comparing the resulting reaction patterns with those observed in red blood cell testing. Agglutination of group A red blood cells indicates the presence in the serum or plasma of anti-A; agglutination of group B red blood cells indicates the presence of anti-B.

The most common forward and reverse group reaction combinations are listed in the table below. A sample with test results that do not match any of the reaction combinations below receives a ??? test interpretation and is considered a No Type Determined (NTD). NTD samples require additional testing which can either be performed on the PK7400, PK7400 or by another method.

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-A,B</th>
<th>A1 Cells</th>
<th>B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AB</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

EXPECTED VALUES

The table below lists the frequencies of the ABO blood groups in the main population groups of the United States.

<table>
<thead>
<tr>
<th>ABO Blood Group</th>
<th>Frequency % in Whites</th>
<th>Frequency % in Blacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>40</td>
<td>27</td>
</tr>
<tr>
<td>B</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>AB</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>O</td>
<td>45</td>
<td>49</td>
</tr>
</tbody>
</table>

XIV. LIMITATIONS OF THE PROCEDURE

As in all blood grouping procedures, contamination of blood specimens, reagent and/or supplementary materials may cause false negative results. In addition, heparin, lithium, citrate, and sodium samples, as well as those containing clots, may cause erroneous results. The reactivity of the product may decrease during the dating period. Use by expiration date.

Chemicals used in the red cell diluent may form crystals when the reagent dries around the threads of the container. To avoid this anomaly, keep the threads of the container free of reagent.

XV. PERFORMANCE CHARACTERISTICS

Specific Performance Characteristics

PK SYSTEM REAGENT RED BLOOD CELLS (A1 and B) meet FDA requirements. There is no U.S. standard of purity, although every lot of product is tested for reactivity and specificity.

Comparison Study

Performance of the PK SYSTEM REAGENT RED BLOOD CELLS (A1 and B) was evaluated during multi-laboratory field trials on PK7400 analyzers by testing randomly chosen samples from normal blood donors in a comparison with FDA-licensed reagent red blood cells.

More than 6,800 samples were tested on the PK7400 analyzers.

The estimated percent agreements on PK7400 testing and their lower limits of 95% one-sided confidence interval for all sites combined are indicated on the table below.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>NPA RQA (LCB)</th>
<th>NPA ROA (LCB)</th>
<th>NP RQA (LCB)</th>
<th>NP ROA (LCB)</th>
<th>PPA RQA (LCB)</th>
<th>PPA ROA (LCB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 Cells</td>
<td>2713</td>
<td>99.79% (98.96%)</td>
<td>4126</td>
<td>100% (99.33%)</td>
<td>5915</td>
<td>99.97% (99.89%)</td>
</tr>
<tr>
<td>B Cells</td>
<td>952</td>
<td>98.75% (97.96%)</td>
<td>5915</td>
<td>99.97% (99.89%)</td>
<td>5915</td>
<td>99.97% (99.89%)</td>
</tr>
</tbody>
</table>

XVI. BIBLIOGRAPHY


XVII. GLOSSARY OF SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT</td>
<td>Batch code</td>
<td>Q</td>
<td>Use by YYYY-MM-DD</td>
</tr>
<tr>
<td>RLU</td>
<td>Catalog number</td>
<td></td>
<td>or YYYY-MM</td>
</tr>
<tr>
<td>EVD</td>
<td>Consult instructions for use</td>
<td>FLD</td>
<td>Storage temperature limitation</td>
</tr>
</tbody>
</table>

XVIII. DATE OF ISSUE

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Technical Service
Phone: 800-447-5352
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