REAGENT RED BLOOD CELLS

A1 Cells / B Cells **REF 17318**

Manufactured by:
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Formulated for Use in Automated Systems
Beckman Coulter PK Systems

141EN01 : May 2020

Distributed by:
BECKMAN COULTER, INC.
250 S. Kraemer Blvd.
BREA, CA 92821 USA

- For *In Vitro Diagnostic Use*
- No U.S. standard of potency
- Do not use if hemolyzed
- Do not freeze
- Preservative: 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 on the surface of human red blood cells and detecting the presence or absence of anti-A and/or anti-B antibodies in the plasma. It is therefore appropriate to identify the red blood cell antigens using known anti-A, anti-B and anti-A,B reagents (red blood cell 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 A1 and B (plasma or reverse group). Discrepancies should be resolved before final interpretation of the ABO group.

THE PRINCIPLE ANTIGENS AND ANTIBODIES OF THE ABO SYSTEM

ABO Blood Group	Antigen present on the red blood cells	Antibodies regularly present in the serum/plasma
O	neither A or B	anti-A and anti-B
A	A	anti-B
B	B	anti-A
AB	A and B	none

III. 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.

IV. REAGENTS

The *PK SYSTEM REAGENT RED BLOOD CELLS* is a suspension of pooled red blood cells. Each bottle contains either group A_1 or B red blood cells. *The NTD rate may be higher due to the use of Rh-positive blood in the group B Reagent Red Blood Cells.* The red blood cells are resuspended to a concentration of 2% (+/- 0.5) in Alsever's solution containing neomycin sulfate, gentamycin sulfate, thiamphenicol and sulfathiazole as preservatives.

The reagents are supplied in 20 mL ready to use plastic vials for the PK7300 and PK7400. *Once opened, the contents of the reagent container may be used until the expiration date on the container.* Store vials in an upright position when not in use. Do not freeze. Do not use if markedly hemolyzed, there is significant darkening of the red blood cells, or if particulate matter is present.

V. WARNINGS AND PRECAUTIONS

1. *CAUTION: 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 ACCORDANCE WITH CURRENT FDA REQUIRED TESTS. NO KNOWN TEST METHOD CAN OFFER 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 a 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 no type determined (NTD) rate.
16. For *in-vitro* diagnostic use.

VI. REAGENT PREPARATION

1. These reagents are intended for use as supplied. No prior preparation or dilution of the reagents is required or permitted.
2. All reagents should be brought to room temperature (+15°C to +30°C) before use on the analyzer. Red blood cells should be completely resuspended by gentle mixing before use on the analyzer.
3. The date on which any reagent container is opened should be recorded on the container.
4. Effort should be made to minimize contamination and prevent evaporation during use of the product.
5. Do not pool reagents in or between vials in any manner. Do not transfer reagent from a new vial to an opened vial. Do not transfer reagent from an open vial to any other container.

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 the 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:
    - Examine for clots prior to 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 1000 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*, A_1 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)
- Physiologic (0.85-0.9%) saline for plasma sample diluent.
  Note: Phosphate 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 plasma 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

Parameter	**Setting**
Sample Volume	120 µL
Diluent Volume	132 µL
Sample/Diluent Ratio	2.1
Diluted Sample Volume	25 µL
Reagent Volume	25 µL for both A_1 and B cells
Channel Name	Variable
Channel Designation	1-12
Decision Logic/Rules	+/-
Temperature Setting	28° C
Incubation Time	60 minutes
Microplate Well	16 µm
Dynamic Range SPC	Low 0, Low 99
Dynamic Range P	Low 45, High 87
Dynamic Range C	Low 0, Low 99
Dynamic Range LIA	Low 0, Low 920
Threshold SPC	Low 11, High 11
Threshold P/C	(+) Limit 24, (-) Limit 20
Threshold LIA	(+) Limit 300, (-) Limit 100
LIA Selection	5
BG/C Limit	High

PK7300 OPERATING INSTRUCTIONS

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

PK7400 RECOMMENDED PARAMETERS

Parameter	Setting
Sample Volume	120 µL
Diluent Volume	132 µL
Sample/Diluent Ratio	2.1
Diluted Sample Volume	25 µL
Reagent Volume	25 µL for both A_1 and B cells
Channel Name	Variable
Channel Designation	1-12
Decision Logic/Rules	+/-
Temperature Setting	30° C
Incubation Time	60 minutes
Microplate Well	16 µm
Dynamic Range SPC	Low 0, Low 60
Dynamic Range P	Low 45, High 87
Dynamic Range C	Low 0, Low 99
Dynamic Range LIA	Low 0, Low 920
Threshold SPC	Low 11, High 11
Threshold P/C	(+) Limit 40, (-) Limit 20
Threshold LIA	(+) Limit 450, (-) Limit 100
LIA Selection	4
BG/C Limit	High

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 diluent

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 be selected to verify positive and negative reactions with every reagent. The positive controls 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 inspected 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 repeatedly, contact BECKMAN COULTER Technical Support at 800-447-5852. 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 chosen for each reagent. 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

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. INTERPRETATON 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,B indicates the presence of the A and/or B antigens, or may suggest that the blood is of a subgroup (such as A_x). Red blood cells of the A_x, and sometimes the A_xB phenotypes may or may not react with Anti-A, depending on the strength to which the antigen is expressed on the particular cells. Most examples of A_x (*i.e*., all besides those having the weakest expression of the antigen) can be expected to react with Anti-A,B in the analyzer.
Confirmation of the test results, is provided by testing the serum or plasma of the blood under investigation with group A_1 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_1 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 PK7300, PK7400 or by another method.

Blood Group	Anti-A	Anti-B	Anti-A,B	A_1 Cells	B Cells
A	+	-	+	-	+
B	-	+	+	+	-
AB	+	+	+	-	-
O	-	-	-	+	+

EXPECTED VALUES

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

ABO Blood Group	Frequency % in Whites	Frequency % in Blacks
A	40	27
B	11	20
AB	4	4
O	45	49

XIV. LIMITATIONS OF THE PROCEDURE

As in all blood grouping procedures, contamination of blood specimens, reagent and/or supplementary materials may give rise to erroneous test results. In addition, heavily lipemic, icteric or hemolyzed samples, as well as those containing clots, may yield erroneous results.
The NTD rate may be higher due to the use of Rh-positive blood in the group B Reagent Red Blood Cells.
The reactivity of the product may decrease during the dating period.
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 containers free of reagent.

XV. PERFORMANCE CHARACTERISTICS

Specific Performance Characteristics

PK SYSTEM REAGENT RED BLOOD CELLS (A_1 and B) meet FDA requirements. There is no U.S. standard of potency, although every lot of product is tested reactivity and specificity.

Comparison Study

Performance of the *PK SYSTEM REAGENT RED BLOOD CELLS* (A_1 and B) was evaluated during multi-sites field trials on PK7400 analyzer 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 analyzer.

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

Overall Statistical Analysis results of the comparison study- PK7400

Reagent	NN	NPA ROA (LCB)	NP	PPA ROA (LCB)
A1 Cells†	2713	99.78% (99.56%)	4168	100.00% (99.93%)
B Cells‡	962	98.75% (97.99%)	5915	99.97% (99.89%)

NN: Number of Negative Samples
NP: Number of Positive Samples
LCB: Lower 95% Confidence Bound
NPA: Negative Percent Agreement
PPA: Positive Percent Agreement
ROA: Overall Rate Of Agreement

† A_1 cells NPA: 99.78% (LCB 99.56%) due to four (4) false positive reactions on the PK7400, plus one (1) false positive and one (1) false negative reaction with the reference method.
‡ B cells NPA: 98.75% (LCB 97.99%) due to nine (9) false positive and one (1) indeterminate (?) reactions on the PK7400, plus one (1) false positive and two (2) false negative reactions with the reference method.
Percent of Agreement only indicates agreement between methods and does not indicate which method gave the correct result(s).

Precision Study
Precision studies were performed, on the same specimens' panel, using multiple operators, days and runs to confirm repeatability and reproducibility of test results in the same run, day and with the same operator and between runs, days and operators. There were no discordant results; all expected positive and all expected negative test generated unequivocal reactions.

For questions or complaints concerning the use of this product(s), please contact Beckman Coulter Technical Support at 800-447-5852.

XVI. BIBLIOGRAPHY

1. Standards for Blood Banks and Transfusion Services. 31st ed. Bethesda, MD: American Association of Blood Banks, 2018
2. Technical Manual. 19th ed. Bethesda, MD: American Association of Blood Banks, 2018.

XVII. GLOSSARY OF SYMBOLS

Symbol	Definition	Symbol	Definition
LOT	Batch code		Use by YYYY-MM-DD or YYYY-MM
REF	Catalog number		Storage temperature limitation
	Consult instructions for use	IVD	*In vitro* diagnostic medical device

XVIII. DATE OF ISSUE

May 2020

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