

REAGENT RED BLOOD CELLS**A₁ and B**

Formulated for Use in Automated Systems
 OLYMPUS® PK® SYSTEMS



Manufactured by
 DIAGAST
 BP 9 – 59374 LOOS CEDEX – FRANCE

U.S. License No: **1744**

Package insert updated in December, 2007 : **057EN01**

I. INTENDED USE

The OLYMPUS® PK® SYSTEM REAGENT RED BLOOD CELLS (A₁ and B) are intended for the determination of the reverse or plasma group on the OLYMPUS PK7200 and PK7300 Automated Analyzers.

II. SUMMARY OF TEST

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 |
|-----------------|--|---|
| O | Neither A nor 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 PK7200 and PK7300 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 OLYMPUS PK7200 refer to the Operator's Manual, and to the User's Guide for the OLYMPUS PK7300 automated analyzer(s).

IV. REAGENTS

Olympus PK System Reagent Red Blood Cells is a suspension of pooled red blood cells. Each bottle contains either group A₁ 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 30 ml plastic vials for the PK7200 and 20 ml ready to use plastic vials for the PK7300. *Once opened, the contents of either the PK7200 or PK7300 reagent container may be used until the expiration date on the container.* Do not use past the expiration date. Store at 2° C to 8° C when not in use. PK Reagent Red Blood Cells left at room temperature for 12 hours or more should be discarded. 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: OLYMPUS 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 PK7200 Operator's Manual and the PK7300 User's Guide.
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 package insert 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. Do not transfer reagent back into the original container or between containers once dispensed or placed into use on the analyzer.
13. Reagents should not be used past the expiration date.
14. 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 - + 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 transfer reagents back into the original container or between containers once dispensed or put into use.

VII. STORAGE

1. Store reagents at 2° C to 8° C when not in use. PK Reagent Red Blood Cells left at room temperature for 12 hours or more should be discarded. Store vials in an upright position when not in use. Do not freeze.
2. Do not use beyond the expiration date.

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 PK7200 and/or PK7300. 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 – 30° C) prior to analysis. Testing should be carried out within five (5) days of collection (see Limitations of the Procedure).
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 PK7200 and/or PK7300. False-positive results may be observed in tests involving the plasma from the sample if particulate matter is not removed during 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 PK7200 and/or PK7300.
- 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 Operator's Manuals for the PK7200 and the User's Guide for the PK7300.

IX. DIRECTIONS FOR USE

MATERIALS PROVIDED:

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

MATERIALS REQUIRED BUT NOT PROVIDED:

- OLYMPUS PK7200 and/or PK7300 Automated Microplate System(s)
- OLYMPUS terraced microplates
- Transfer pipettes or equivalent
- Centrifuge
- Control samples (positive and negative)
- Mixing comb (PK7200 only)
- Physiologic (0.85-0.9%) saline for making plasma dilutions.

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

The PK7200 and PK7300 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 PK7200 and PK7300 is shown below.

| Parameter Settings for the PK7200 and PK7300 for A ₁ and B Reverse Grouping Cells | | |
|---|---|---|
| Parameter | Settings | |
| | PK7200 | PK7300 |
| Sample Volume | 200-250 µL | 120 µL |
| Diluent Volume | 250 µL (Stroke Pin G 0.25) | 132 µL |
| Sample/Diluent Ratio | 800-1000 µL/1000 µL | 2.1% |
| Diluted Sample Volume | 25-30 µL | 25 µL |
| Reagent Volume | 25 µL for both A ₁ and B cells | 25 µL for both A ₁ and B cells |
| Channel Name | Variable | Variable |
| Channel Designation | 1-12 | 1-12 |
| Decision Logic | +/- | +/- |
| Temperature Setting | 28° C | 28° C |
| Incubation Time | 60 minutes | 60 minutes |
| Microplate Well | 16 µm | 16 µm |

| Threshold settings for the PK7200 and PK7300 | | | |
|---|----------|---------------|---------------|
| for A ₁ and B Reverse Grouping Cells | | | |
| Dynamic Range | Setting | Threshold | Setting |
| SPC (7300 only) | Low 0 | SPC | Low 11 |
| | High 99 | | High 11 |
| P | Low 45 | P/C | (+) Limit 24 |
| | High 87 | | (-) Limit 20 |
| C | Low 0 | LIA | (+) Limit 300 |
| | High 99 | | (-) Limit 100 |
| LIA | Low 0 | LIA Selection | 5 |
| | High 920 | BG/C Limit | High |

PK7200 OPERATING INSTRUCTIONS

- Using the reagent and diluent configuration displayed on the TEST REQUISITION screen, add the reagent red blood cells in the appropriate channels of the reagent container using a transfer pipette. *The OLYMPUS PK7200 SYSTEM REAGENT RED BLOOD CELLS are ready for use on the analyzer and should not be altered in any way prior to use.*
- Place the diluent line(s) for ABO reverse group testing into the diluent container filled with physiologic saline (phosphate buffered saline should not be used).
- Place the reagent container and mixing comb (mixing comb required for reagent red blood cells) on the analyzer. Press the R MIX switch on the analyzer to start the motion of the mixing comb if there is any delay in initiating processing.
- Remove the G stroke pins for the diluent lines if a black rack filled with saline tubes is not being processed at the beginning of the run.
- Push the PREP switch on the analyzer.
- When the PREP cycle is complete, place the G stroke pins in the locations indicated on the TEST REQUISITION screen being certain to use the G 0.25 stroke pin for the ABO plasma diluent line.
- Press the DIAG switch on the analyzer control panel to expel bubbles in the reagent, sample and diluted sample probes.
- Proceed with sample analysis as described in Section 7 of the OLYMPUS PK7200 Operator's Manual.

PK7300 OPERATING INSTRUCTIONS

- Using the PANEL configuration screen from the START CONDITION menu, place the reagent containers in the appropriate slots in the reagent tray. *The OLYMPUS PK7300 SYSTEM REAGENT RED BLOOD CELLS are packaged in ready to use containers that are placed directly into the reagent tray and should not be altered in any way prior to use.*
- Place the diluent line for ABO reverse group testing into the diluent container filled with physiologic saline (*phosphate buffered saline should not be used*).
- From the SYSTEM STATUS menu, press the PREPARATION key, then check Diluent Priming and press the YES key.
- After priming is complete, press the REAGENT/DILUENT STATUS key, then press the DILUENT CHECK START which will enable the use of the handy scanner. Scan each diluent position ID label, then the diluent ID label. Press the DILUENT CHECK END key when scanning is completed.
- Press the EDIT key and enter the volume of each diluent.
- Press the REAGENT CHECK START and REAGENT CHECK END keys.
- If no errors are detected in the diluent or reagent areas, the START key may be pressed to begin analysis.
- Proceed with sample analysis as outlined in section C of the OLYMPUS PK7300 User's Guide.

X. QUALITY CONTROL

PK7200 and PK7300

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 OLYMPUS Technical Support at 800-447-5852. Please refer to the PK7200 Operator's Manual and the PK7300 User's Guide for additional information concerning the use of control samples.

XI. INTERPRETATION

The PK7200 and the PK7300 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 12 in the OLYMPUS PK7200 SOP and Section G in the OLYMPUS PK7300 User's Guide for complete details of the manner in which the analyzer interprets reactions.

Within 30 minutes after analyzer interpretation on the PK7200, results should be verified by visual review of the reaction patterns in the microplate wells against the analyzer printout. The PK7300 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.

XII. 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,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 A₁ and group B red blood cells, and by comparing the resulting reaction patterns with those observed in red blood cell testing. Agglutination of group A₁ 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 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 PK7200, PK7300 or by another method.

| Blood Group | Forward Group | | | Reverse Group | |
|-------------|---------------|--------|----------|----------------------|---------|
| | Anti-A | Anti-B | Anti-A,B | A ₁ Cells | B Cells |
| A | + | - | + | - | + |
| B | - | + | + | + | - |
| AB | + | + | + | - | - |
| O | - | - | - | + | + |

XIII. EXPECTED VALUES

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

| ABO Blood Group | Frequency % | |
|--------------------|-------------|--------|
| | Whites | 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. SPECIFIC PERFORMANCE CHARACTERISTICS








OLYMPUS PK SYSTEM REAGENT RED BLOOD CELLS (A₁ and B) meet FDA requirements. There is no U.S. standard of potency.

For questions or complaints concerning the use of this product, please contact Olympus Technical Support at 800-447-5852.

BIBLIOGRAPHY

- Standards for Blood Banks and Transfusion Services. 24th ed. 2006; American Association of Blood Banks: 5.8.2; and, 21 CFR 606.121(c)(12).
- Technical Manual. 15th ed. 2005; American Association of Blood Banks: 322-3323.
- 21 CFR 660.30 – 660.36.

Glossary of Symbols

| Symbol | Definition | Symbol | Definition |
|---|---------------------------------|--|--|
|  | Batch code |  | Use by YYYY-MM-DD or YYYY-MM |
|  | Catalog number |  | Upper limit of temperature |
|  | Consult instructions for use |  | <i>In vitro</i> diagnostic medical device |
|  | Manufacturer | | |



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Package insert updated in December, 2007