



INTENDED USE

American Red Cross (Red Cross) Anti-Kp^b Blood Grouping Reagent is used for testing human red blood cells by the indirect antiglobulin test.

SUMMARY AND EXPLANATION

The Kp^b (K4) blood group antigen was first described in 1958 by Allen, Lewis and Fudenberg¹ and was subsequently assigned to the Kell blood group system. Today, the Kell blood group system has expanded to include more than 20 well defined, discrete antigens. Examples of anti-Kp^b are usually produced in response to immunization by transfusion or pregnancy. They are usually IgG in nature and react primarily by the indirect antiglobulin test (IAT). Anti-Kp^b has been reported to cause transfusion reactions and hemolytic disease of the fetus and newborn (HDFN).

The Kp^b antigen is found in >99.9% of Caucasians, Blacks and Oriental people of eastern Asia and America.¹

PRINCIPLE OF PROCEDURE

American Red Cross (Red Cross) Anti-Kp^b reacts optimally by the indirect antiglobulin test after a 37 C incubation with washed red blood cells. Following incubation, the red blood cells are washed free of unbound serum proteins and an Anti-Human Globulin reagent is added. Agglutination indicates the presence of the Kp^b antigen on the red blood cells and is interpreted as a positive result. Lack of agglutination indicates the absence of the Kp^b antigen and is interpreted as a negative result.

REAGENT

Each lot of Red Cross Anti-Kp^b is prepared from a pool of human sera that has been processed to remove unwanted antibodies and provide a potent and specific reagent that meets or exceeds the requirements of the Food and Drug Administration (FDA). Each lot is standardized for pH and protein concentration, and may contain sodium chloride, sodium or potassium phosphates, bovine albumin, and high molecular weight polymers to enhance agglutination. The bovine albumin component of this product is derived exclusively from United States sources of disease-free cattle, inspected and certified by the US Veterinary Services. This ruminant-based product is deemed to have low Transmissible Spongiform Encephalopathy (TSE) risk. The total protein concentration is 3-7%. The product contains sodium azide (final concentration of 0.1%) as a preservative.

Red Cross Anti-Kp^b is for *in vitro* diagnostic use and is supplied ready for use, no dilution or modification is required.

Rx Only

CAUTION: All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.

Caution: This Product Contains Natural Rubber Latex (Dropper Bulbs) Which May Cause Allergic Reactions.

Warning: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into sinks, flush with a large volume of water to prevent azide build-up.

STORAGE

Store at 1-8 C when not in use.

Even though this product is clear following filtration, soluble lipoproteins may precipitate over the dating period. Adsorptions during the manufacturing process may cause the reagent to appear dark in color. These conditions are not signs of deterioration. Deterioration is recognized through serologic testing ordinarily performed routinely with control red blood cells.

Bacterial or fungal contamination may cause erroneous results.

Do not use beyond expiration date. The format for the expiration date is expressed as YYYY-MM-DD (year month day).

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient or donor is required prior to specimen collection. Clotted blood or blood drawn into an approved anticoagulant may be used. Blood drawn into heparin or oxalate should be tested within 2 days. Clotted samples or blood drawn in EDTA or sodium citrate should be tested within 14 days. Donor blood may be tested up to the expiration date. Store specimens at 1-8 C when not required for testing. Storage may result in weaker-than-normal reactions.

MATERIALS

Materials provided:

1. American Red Cross Anti-Kp^b

Materials required but not supplied:

1. Test tubes, 10 x 75 mm or 12 x 75 mm
2. Test tube racks
3. Pipettes
4. Centrifuge
5. Isotonic saline (pH 6.0 – 7.5)
6. Incubating and support equipment
7. Antigen positive and negative control cells
8. Anti-Human Globulin containing anti-IgG
9. Antiglobulin control cells (IgG sensitized red blood cells)
10. Timer
11. Optical aid (optional)

PROCEDURE

1. Prepare a 2-4% suspension of red blood cells washed at least once with isotonic saline.
2. Add 1 drop of Red Cross Anti-Kp^b to an appropriately labeled test tube.
3. Add 1 drop of the previously prepared 2-4% red blood cell suspension.
4. Mix well.
5. Incubate the tube at 36-38 C for 15-30 minutes.
6. Wash the red blood cells at least 3 times with isotonic saline. Ensure that all the saline is decanted and that the red blood cells are resuspended between washes.
7. Add Anti-Human Globulin containing anti-IgG according to the manufacturer's directions. (Anti-IgG or polyspecific Anti-Human Globulin may be used in the test system.)
8. Mix well and centrifuge the tube for 15 seconds at 3400 rpm (900-1000 rcf) or 1 minute at 1000 rpm (100-120 rcf) or equivalent, as indicated by equipment quality control calibration.
9. Resuspend the red blood cells by gentle agitation.
10. Read macroscopically for agglutination and record results. An optical aid may be used in reading, if desired.
11. Add antiglobulin control cells to all negative tests and centrifuge as above. Agglutination of the antiglobulin control cells confirms the presence of active anti-IgG. No agglutination of the antiglobulin control cells may indicate that the antiglobulin reagent has been neutralized or omitted and that tests should be repeated.

$$*rcf=0.00001118 \times \text{radius (cm)} \times (\text{rpm})^2$$

STABILITY OF REACTION

Tests should be read and results recorded immediately following centrifugation. Delays may result in dissociation of antigen-antibody complex and result in weaker or, in some cases, false negative results.

Consideration must be given to the time it takes to process, read and record each group of tests. It is the responsibility of the user to determine the most practical number of samples that can be tested at one time that provides for a consistent test process and keeps delays at a minimum.

QUALITY CONTROL

Controls of known antigen positive cells, preferentially with heterozygous expression, and known antigen negative cells should be used to confirm reactivity of the reagent on each day of use.

All negative test results should be tested with antiglobulin control cells (IgG sensitized red blood cells).

INTERPRETATION OF RESULTS

Agglutination of the red blood cells is a positive (+) test result and indicates the presence of the Kp^b antigen. No agglutination and correct performance with antiglobulin control cells is a negative (-) test result and indicates the absence of the Kp^b antigen. Hemolysis, if obtained, should not be interpreted as a positive result since the conditions for complement activation due to a red blood cell antibody-antigen reaction do not exist.

LIMITATIONS

All serological tests have limitations. To maximize success in obtaining valid results, follow the directions for use carefully. Deviations from manufacturer's instructions without appropriate validation and controls may produce erroneous results.

- Red blood cells must be washed with adequate volumes of isotonic saline to avoid possible neutralization of Anti-Human Globulin reagent by residual serum proteins.
- Resuspend red blood cells in isotonic saline or other appropriate solution. The pH of saline may affect the stability of the reaction. It is recommended that the pH be 6.0 - 7.5.

Erroneous results may also be caused by:

- Aged blood specimens yielding weaker reactions.
- Cell suspensions outside of the recommended concentration.
- Red blood cells with a positive direct antiglobulin test.
- Microscopic examination of tests.
- Contaminated samples or materials.
- Bacterial or fungal contamination.
- Some drug/disease states.
- Improper centrifugation. Centrifugation equipment should be calibrated and checked regularly to insure correct operation.
- Improper incubation time and temperature.

Suppressed or weakened expression of blood group antigens may give rise to false-negative reactions or non-concordance with similar reagents and/or alternate methodologies.

SPECIFIC PERFORMANCE CHARACTERISTICS

Red Cross Anti-Kp^b has been manufactured to meet or exceed FDA potency requirements. Each lot is tested against a panel of antigen-positive red blood cells to assure appropriate reactivity when used by the recommended test procedure. The specificity of each lot is verified by the recommended tube method using a panel of red blood cells which lack the antigen against which the reagent is directed, but carry as many other antigens as possible having a frequency of 1% or greater. When direct exclusion is not possible, e.g., due to rarity of red blood cells, contaminants may be excluded by selective adsorption techniques.

Specificity test results submitted to the FDA for release of an individual lot of product will be furnished upon request.

For technical questions, contact the American Red Cross Diagnostic Manufacturing Division at 1-800-882-3737.

BIBLIOGRAPHY

1. Issitt PD, Anstee DJ. *Applied Blood Group Serology*, 4th ed. Durham, NC; Montgomery Scientific Publications, 1998.
2. Fung, Mark K., ed. *Technical Manual*, 18th ed. Bethesda, MD; AABB, 2014.

For Optional Use by User:

Lot Number: _____

Expiration: _____

The American National Red Cross
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