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BLOOD GROUPING REAGENT

Anti-k̄ (Monoclonal) (IgG)

IVD *In vitro* diagnostic medical device

American Red Cross

INTENDED USE

American Red Cross (Red Cross) Anti-k̄ (Monoclonal) (IgG) is used for the *in vitro* detection of the k̄ (KEL2) antigen on human red blood cells by the Indirect Antiglobulin Test (IAT).

SUMMARY AND EXPLANATION

Since the discovery of the k̄ blood group antigen, and its association to the Kell system in 1949 by Levine *et al*, the Kell system classification has expanded to include more than 36 antigens/phenotypes.[1-3] Anti-K and Anti-k̄ may cause severe transfusion reactions and hemolytic disease of the fetus and newborn (HDFN) and therefore the antigens are important in pre-transfusion testing and the prediction of HDFN.

The prevalence of the K and k̄ antigen varies in different populations, as shown in Table 1:

Reagent		Phenotype	Prevalence[3] (%)	
Anti-K	Anti-k̄		Whites	US Blacks
0	+	K- k̄ +	91	98
+	+	K+ k̄ +	8.8	2
+	0	K+ k̄ -	0.2	Rare

PRINCIPLE OF PROCEDURE

When the Directions for Use (DFU) are followed, this reagent reacts optimally by the indirect antiglobulin test after a 36ºC to 38ºC 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 k̄ antigen (positive test). Lack of agglutination indicates the absence of the k̄ antigen (negative test).

REAGENT

Red Cross Anti-k̄ (Monoclonal) (IgG) is prepared from human IgG antibodies derived from cell culture supernatant of the human/murine heterohybridoma cell line P3A118OL67. The antibodies are immunoglobulin class IgG and provide a potent and specific reagent that meets the requirements of the Food and Drug Administration (FDA). Each lot is optimized for tube indirect antiglobulin testing and standardized for pH and total protein concentration in a buffered diluent containing macromolecular chemical potentiators. The bovine albumin component of these products is derived exclusively from United States sources of disease-free cattle, inspected, and certified by the U.S. Veterinary Services. This ruminant-based product is deemed to have low Transmissible Spongiform Encephalopathy (TSE) risk. The reagents contain sodium azide (0.1% final concentration) as a preservative.

These monoclonal antibodies are manufactured using intermediate products produced for the Red Cross in a shared manufacturing agreement with Millipore (UK) Ltd., Fleming Road, Kirkton Campus, EH547BN, Livingston, UK; U.S. License Number 1761.

Red Cross Anti-k̄ (Monoclonal) (IgG) is for *in vitro* diagnostic use and are supplied ready for use. Use as furnished, do not dilute.

Red Cross Monoclonal Blood Grouping Reagents meet FDA potency requirements.

CAUTION STATEMENTS
Rx only

CAUTION: 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 methods can offer assurance that products derived from hybridoma cell culture supernatant will not transmit infectious agents. The absence of murine virus has not been determined.

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 2ºC to 8ºC when not in use.

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

Do not use if turbid.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient or donor is required prior to specimen collection. Blood should be collected by an acceptable phlebotomy technique. Samples may be drawn into EDTA, CPD, CPDA-1, CPD with AS-1, CP2D with AS-3, and CPD with AS-5, or may be drawn without anticoagulant (non-barrier red top). Do not use samples drawn into tubes with neutral gel separators.

Samples should be tested as soon as possible after collection. Samples should be stored at 2ºC to 8ºC when not required for testing.

Blood drawn into EDTA, and non-barrier red top (clotted) tubes should be tested less than or equal to 10 days of collection. Donor samples drawn into CPD, CPDA-1, CPD with AS-1, CP2D with AS-3, and CPD with AS-5 may be tested up to the expiration date of the donor unit.

NOTE: Due to the rarity of the k̄ - phenotype, Red Cross was unable to evaluate the use of Red Cross Anti-k̄ (Monoclonal) (IgG) with CPDA-1, CP2D with AS-3, and CPD with AS-5 samples from k̄ negative individuals.

MATERIALS

Materials provided:

1. Red Cross Anti-$\overline{k}$ (Monoclonal) (IgG)

Materials required but not supplied:

1. Test tubes, 10 x 75mm or 12 x 75mm
2. Test tube racks
3. Pipettes
4. Centrifuge calibrated for serological use
5. Isotonic Saline (pH 6.5 – 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-$\overline{k}$ (Monoclonal) (IgG) reagent 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 at 36ºC to 38ºC for 15 minutes.
6. Wash the red blood cells at least 3 times with isotonic saline, thoroughly decanting the saline.
7. Add 2 drops of Anti-Human Globulin reagent, according to the manufacturer's directions.
8. Mix well and centrifuge tubes for 15 seconds at 3400 rpm (900-1000 rcf*) or equivalent, as indicated on the 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 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 the test is invalid and should be repeated.

*rcf=0.00001118 x radius (cm) x $(rpm)^2$

STABILITY OF REACTION

Following centrifugation, all tube tests should be read/recorded without delay. Time delays may cause a dissociation of the antigen-positive complexes resulting in false negative or weaker than expected reactions.

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

The reactivity of this reagent should be confirmed on each day of use by testing with known antigen-positive (preferably heterozygous expression) and with known antigen-negative red blood cells. The reagent is acceptable for use if it reacts only with the antigen-positive red blood cells.

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

INTERPRETATION OF RESULTS

Positive (+) test result: visible agglutination of the red blood cells after the addition of the antiglobulin reagent and centrifugation.

NOTE: Hemolysis, if obtained, should not be interpreted as a positive result since the conditions for complement activation due to a red cell antibody-antigen reaction do not exist.

Negative (-) test result: no agglutination of red blood cells after the addition of the antiglobulin reagent, centrifugation, and correct performance with antiglobulin control cells.

LIMITATIONS

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

False positive results are possible when testing a sample with a positive direct antiglobulin test (DAT). The inclusion of an auto-control is recommended in this circumstance or if this circumstance is suspected.

False positive or false negative test results may occur from bacterial or chemical contamination of test materials, improper incubation temperature or time, centrifugation errors, storage temperatures excursions, or omission of test reagents.

The resuspension of the button in serological reactions in the tube test procedure must be carried out by using gentle agitation. Shaking too strongly may cause agglutinates to be dispersed.

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. The presence of the Kp^a antigen may result in the diminished expression of $\overline{k}$. Notably, red blood cells that are both K+ and Kp(a+) may show a weaker reaction when compared to the red blood cells chosen for the positive control test. In addition, the rare McLeod phenotype also is known to result in very weak expressions of Kell system antigens.[3]

SPECIFIC PERFORMANCE CHARACTERISTICS

Red Cross Anti-$\overline{k}$ (Monoclonal) (IgG) has been manufactured to meet FDA potency requirements. Each lot is tested against a panel of antigen-positive red blood cells (heterozygous expression and/or weakened expression if possible) to ensure appropriate reactivity when used by the recommended test procedure in the DFU. The specificity of each lot is verified by the recommended tube testing method using a panel of well characterized red cells that lack the reagent antigen.

PERFORMANCE CHARACTERISTICS BY MANUAL TUBE METHOD:

The performance of Red Cross Anti-$\overline{k}$ (Monoclonal) (IgG) was confirmed against an FDA licensed reference reagent (comparator) in a multi-center field trial, representing blood collection establishments, transfusion services and/or immunohematology reference laboratories which tested donor, patient, and neonatal samples. The testing sites were selected to cover diverse geographic locations and patient/donor populations.

Table 2

N=1519		Comparator Reagent			
		Positive	Negative		
Red Cross Anti-$\overline{k}$ Lot LK-2	Positive	1500	0	Positive Percent Agreement	100.00%
				PPA (95% 1-Sided LCI)	99.80%
	Negative	0	19	Negative Percent Agreement	100.00%
				NPA (95% 1-Sided LCI)	85.41%*

*The PPA met the acceptance criterion. The NPA was at 85.41% due to the low frequency of antigen negative samples in the population.

Table 3

N=1519		Comparator Reagent			
		Positive	Negative		
Red Cross Anti-$\overline{k}$ Lot LK-3	Positive	1500	0	Positive Percent Agreement	100.00%
				PPA (95% 1-Sided LCI)	99.80%
	Negative	0	19	Negative Percent Agreement	100.00%
				NPA (95% 1-Sided LCI)	85.41%*

*The PPA met the acceptance criterion. The NPA was at 85.41% due to the low frequency of antigen negative samples in the population.

PRECISION STUDY RESULTS

As part of the performance evaluation, a precision and reproducibility study was performed using multiple operators at multiple sites on 5 non-consecutive days over a 20-day period. Testing confirmed repeatable and reproducible results within each run, run-to-run, day-to-day, operator-to-operator, and site-to-site. There were no discordant results.

A lot-to-lot study was also performed using three lots of each specificity on 5 non-consecutive days over a 20-day period which confirmed repeatable and reproducible results within each test run, run-to-run, and day-to-day. There were no discordant results.

Specificity tests 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.

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1. Levine P., Backer M., Wigod M., and Ponder RA. Science. 1949; 109: 464-466.
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