BLOOD GROUPING REAGENT

Anti-$\bar{k}$

By Indirect Antiglobulin Test

IVD

Rx ONLY

1°C–10°C

Harmful, Preservative: 0.1% Sodium Azide Meets FDA Potency Requirements

CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. THE PACKAGING OF THIS PRODUCT (DROPPER BULB) MAY CONTAIN DRY NATURAL RUBBER.

Immucor, Inc.
3130 Gateway Drive
Norcross, GA 30071 USA
US LICENSE 886

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EC REP
Immucor Medizinische Diagnostik GmbH
Robert-Bosch-Strasse 32
63303 Dreieich, GERMANY

BLOOD GROUPING REAGENT

Anti-$\bar{k}$

By Indirect Antiglobulin Test

IMMUCOR

INTENDED USE: Immucor Anti-$\bar{k}$ Blood Grouping Reagent is intended for the detection of the k (KEL2) antigen on red blood cells by the indirect antiglobulin test.

SUMMARY OF THE TEST: Since the discovery of the first Kell system antibody, anti-Kell (now called anti-K), by Coombs, Mourant and Race in 1946 [1], the system has grown to become almost as complex as Rh. Anti-Cellano (anti-$\bar{k}$) the antibody antithetical to anti-K was reported in 1949 by Levine and his co-workers [2]; and in 1957 and 1958, Allen and associates [3,4] reported a second pair of antithetical antibodies, anti-Penney (anti-Kp^a) and anti-Rautenberg (anti-Kp^b). The system was further expanded in 1965, when Stroup and associates [5] recognized that anti-Sutter (anti-Js^a) and anti-Matthews (anti-Js^b), which had been reported, respectively, by Giblett [6] in 1958 and Walker *et al.* [7] in 1963, were defining a third pair of Kell-related alleles.

The Js^a (KEL6) antigen is predominantly a characteristic found in persons of African descent, while Kp^a (KEL3) appears to be confined to Whites, and the K (KEL1) antigen has a higher prevalence among Whites than among African Americans.

The six antigens mentioned are the most significant ones of the Kell system for the routine hospital blood bank, although others have been described. The interested reader is referred for further information to the appropriate chapter of *the Blood Group Antigen FactsBook, by Reid and Lomas-Francis* [8].

Immucor Anti-$\bar{k}$ Blood Grouping Reagent is used to detect the presence of the k antigen on donor or patient red blood cells. Typing of donor red blood cells facilitates the selection of antigen-negative units for transfusion to patients with the corresponding antibody. Red blood cell typing also serves as final verification of the identification of an alloantibody in patient or donor serum.

PRINCIPLE OF THE TEST: The presence of the k antigen is determined by testing with Anti-$\bar{k}$ by the indirect antiglobulin technique. Agglutination of the test red blood cells constitutes a positive test result and indicates the presence of the k antigen. No agglutination constitutes a negative test result and indicates that the k antigen is not present.

REAGENT DESCRIPTION: Blood Grouping Reagent, Anti-$\bar{k}$ as supplied by Immucor, is prepared from human blood containing appropriate antibodies, active by the indirect antiglobulin technique. The final formulation includes bovine albumin in a sodium chloride solution. Any bovine albumin used in the manufacture of this product is sourced from donor animals of United States origin that have been inspected and certified by USDA Food Safety and Inspection Service inspectors to be disease-free. This ruminant-based product is deemed to have low TSE (Transmissible Spongiform Encephalopathy) risk. Glycine may also be present. Contains 0.1% sodium azide as a preservative.

PRECAUTIONS:

For in vitro diagnostic use. Store at 1° to 10°C when not in use. Do not freeze. Do not dilute. Do not use past the expiration date. Effort should be made to minimize contamination during use of the product. These products are clear after filtration, but may develop turbidity over the dating period, due to the precipitation of soluble lipoproteins. Turbidity due to this cause is not an indication of product deterioration. Serological testing is required to recognize product deterioration. This would ordinarily be accomplished in the course of routine testing with control red blood cells.

This reagent contains 0.1% sodium azide. Warning: EUH032 Harmful if swallowed.

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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.

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 HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS. THE PACKAGING OF THIS PRODUCT (DROPPER BULB) MAY CONTAIN DRY NATURAL RUBBER.

Handle and dispose of reagent as potentially infectious.

The format for the expiration date is expressed as CCYY-MM-DD (year-month-day).

SPECIMEN COLLECTION AND PREPARATION: No special preparation of the patient is required prior to specimen collection. Blood should be drawn by aseptic technique, with or without an anticoagulant. The specimen should be tested as soon as possible after collection. If delay in testing should occur, the specimen must be stored at 1° to 10°C. Bacterial contamination of the specimen may cause false test results. Blood drawn into EDTA should not be stored for longer than seven days. It is best to test oxalated or heparinized blood samples within two days of collection. Clotted specimens may be tested up to 21 days after collection, and donor blood may be tested up to the expiration date. Storage may result in weaker-than-normal reactions.

PROCEDURE:

Materials Provided: Immucor Anti-$\bar{k}$

Materials and Equipment Not Supplied: Test tubes (12 × 75mm or 10 × 75 mm), pipettes, isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline pH 6.5-7.5, 37°C waterbath or incubator, timer, centrifuge, and an optical aid such as a hand lens or a concave mirror. Anti-Human Globulin containing anti-IgG, IgG-sensitized red blood cells, and red blood cells of known Kell phenotypes for use as controls.

TEST METHOD:

1. Place 1 or 2 drops of the Immucor Anti-$\bar{k}$ in a properly labeled test tube. Adding 2 drops of reagent may enhance reactivity.
2. Add 1 drop of an approximate 3-4% suspension of the red blood cells to be tested, previously washed at least one time and resuspended in saline.
3. Mix well by shaking the tube and incubate for 15 to 30 minutes at 37°±1°C. Incubating for the upper end of the time range may enhance reactivity.
4. Wash the red blood cells at least 3 times with the tubes full of saline, being careful to decant the saline between washes and to resuspend the red blood cells thoroughly when adding saline for the next wash. Decant the saline completely following the last wash.
5. Add 1 or 2 drops of Gamma-clone® Anti-Human Globulin to each “dry button” of red blood cells, or follow the directions of the Anti-Human Globulin manufacturer. Adding 2 drops of AHG may enhance reactivity.
6. Mix well and centrifuge for:
   (a) 1 minute at 1,000 rpm (rcf 100 to 125) or
   (b) 15 seconds at 3,400 rpm (rcf 900 to 1,000) or
   (c) a time appropriate to the calibration of the centrifuge.

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7. Resuspend the red blood cells by gentle shaking and examine for macroscopic agglutination. Negative reactions may be examined with an optical aid; however, microscopic reading is not recommended. Record results.

Stability of Reaction: The washing phases of the antiglobulin test must be carried out without interruption, and final test results must be interpreted immediately upon completion of the test.

QUALITY CONTROL:

1. All negative tests should be confirmed by adding IgG-sensitized red blood cells, such as Checkcell®, and then repeating centrifugation and reading. A positive test result at this point confirms that active antiglobulin (anti-IgG) was added to the test system and was present when the original test was interpreted as negative.
2. The reactivity of blood grouping reagents should be confirmed on each day of use by testing with red blood cells known to be negative and positive for the relevant antigens. K+k+ red blood cells are the most suitable positive control red blood cells for Anti-k.
3. It is necessary to carry out a direct antiglobulin test on each red blood cell suspension being typed, to confirm that any agglutination is truly due to an antigen-antibody reaction between the test red blood cells and the blood grouping reagent. This control may be omitted if the tests are negative or if the red blood cells are being typed by the indirect antiglobulin technique with blood grouping reagents of other specificities and yield a negative result.

INTERPRETATION OF TEST RESULTS: Agglutination of the red blood cells constitutes a positive test result and indicates the presence of the relevant antigen, providing the test red blood cells do not have a positive direct antiglobulin test.
No agglutination constitutes a negative test result, and indicates the absence of the relevant antigen.
The reaction patterns of Kell system antibodies are shown in Table 1, together with the frequencies of the resulting phenotypes in the US population.

Anti-				Phenotype	Prevalence %	
-K	-k	-Kp^a	-Kp^b		Whites	African Americans
+	0			K+k–	0.2	rare
+	+			K+k+	8.8	2
0	+			K–k+	91	98
		+	0	Kp(a+b–)	rare	0
		+	+	Kp(a+b+)	2.3	rare
		0	+	Kp(a–b+)	97.7	100
0	0	0	0	K_o	exceedingly rare	

Table 1. The reaction patterns encountered when testing blood samples for the main antigens of the Kell blood group system, together with the approximate frequencies of the resulting phenotypes in the US population [10].

As in all blood grouping tests, diminished antigen expression may be a source of false test results if a weak reaction is interpreted as negative. A feature of the rare McLeod phenotype [11], which has been observed in some cases of chronic granulomatous disease [12], is a very weak expression of Kell system antigens. In addition, the presence of the Kp^a antigen may be accompanied by diminished expression of k. In particular, red blood cells that are both K+ and Kp(a+) may show a substantially weaker reaction than the red blood cells chosen for the positive control test.

LIMITATIONS: Antibodies directed at blood group antigens of low incidence sometimes occur as unsuspected contaminants in blood grouping reagents. In addition, certain antigens (e.g. Bg, Sd^a) can be present in an exalted state on the red blood cells. These phenomena may be a source of rare false-positive reactions, which may occur with more than one lot of a given specificity. Since manufacturers commonly obtain raw material from the same sources, the same contaminating antibody may be present in products acquired from different manufacturers. It is not possible for any manufacturer to claim the absence of all contaminating antibodies, as red blood cells carrying antigens of low-incidence and exalted antigens are not always available for testing. Suppressed or diminished expression of certain blood group antigens may conversely give rise to substantially weaker reactions than are observed with the red blood cells normally used for the positive control test. For these reasons, caution should always be exercised when assigning genetic significance on the basis of test results.

Other factors that may cause false test results include the following:

1. Contamination of blood specimens, reagent and/or supplementary materials.
2. Aged blood specimens, which may yield weaker reactions than those obtained with fresh red blood cells.
3. Too heavy a red blood cell suspension.
4. Improper incubation time or temperature.
5. Improper centrifugation. Excessive centrifugation may lead to difficulty in resuspending the red blood cell button in the tube test. At the same time, inadequate centrifugation may yield unclear red blood cell button patterns and agglutinates that are too readily dispersed.
6 Improper examination for agglutination (usually too vigorous shaking). The resuspension of reactions in the tube test procedure must be carried out by gentle shaking. Shaking too vigorously may cause agglutinates to be dispersed.
7. Red blood cells having a positive direct antiglobulin test due to coating of IgG cannot be typed by the indirect antiglobulin technique.

SPECIFIC PERFORMANCE CHARACTERISTICS: Immucor Anti-$\overline{k}$ Blood Grouping Reagents meet FDA potency requirements. Each lot is tested against at least 10 red blood cell samples positive for the k antigen to assure adequate reactivity in use. All lots are additionally tested against a panel of selected red blood cell samples negative for the k antigen. The panel includes red blood cells positive (or presumptively positive) for antigens having a prevalence of 1% or more in the general population of the US. Antibodies to Le^c and Le^d are not necessarily excluded, nor are antibodies to such low-incidence antigens as Wr^a, M^g, Di^a and Vw, although testing for such contaminants may be undertaken if suitable test red blood cells are available. The absence of antibodies to V and VS is confirmed. Confirmation of the absence of contaminating antibodies is difficult, due to the high frequencies of the respective specific antigens and to the resulting scarcity of suitable test red blood cells. In testing lots of these products for specificity, the number of antigen-negative red blood cells available for direct testing may be limited to four, and prior adsorption of the specific antibody may be required to allow tests to be carried out with red blood cells positive for the antigens referenced above. The performance of this product is dependent on adhering to the recommended methods found in this insert.

For additional information or for technical support, contact Immucor at 855-IMMUCOR (466-8267).

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