BLOOD GROUPING REAGENT

Anti-$\bar{k}$ (Monoclonal) (IgG)

Gamma-clone®

By Indirect Agglutination Test

Preservative: 0.09% Sodium Azide   Meets FDA Potency Requirements IVD

CAUTION: THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) MAY CONTAIN DRY NATURAL RUBBER. DO NOT PIPETTE THIS PRODUCT BY MOUTH, AS THE ABSENCE OF MURINE VIRUS HAS NOT BEEN DETERMINED.
DO NOT USE IF MARKEDLY TURBID.

Rx ONLY

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IMMUCOR

Intended Use:

Gamma-clone Anti-$\bar{k}$ (Monoclonal) (IgG) Blood Grouping Reagent is intended for the detection of the k (KEL2) antigen on red blood cells by indirect agglutination tube 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].

Gamma-clone Anti-$\bar{k}$ (Monoclonal) (IgG) 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 relevant antigen. No agglutination constitutes a negative test result and indicates that the antigen is not present.

Reagents:

Gamma-clone Anti-$\bar{k}$ (Monoclonal) (IgG) Blood Grouping Reagent is prepared from human IgG antibodies from the hybridoma cell line P3A118OL67 grown in fluid culture and suitably diluted in a proprietary diluent containing bovine albumin to achieve the appropriate level of potency for the test procedure as described. Sodium azide is added as a preservative (at 0.09% w/v). Ready for use as supplied.

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 a low-TSE (Transmissible Spongiform Encephalopathy) risk.

Storage:

- Store at 1ºC to 10ºC when not in use.
- Do not use beyond the expiration date which is expressed as CCYY-MM-DD (year-month-date).
- Do not freeze.

Precautions:

- For *in vitro* diagnostic use.
- Do not dilute.
- Effort should be made to minimize contamination during use.
- Do not use if markedly turbid.

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Sodium azide is added as a preservative (at 0.09% w/v). Waste fluids arising from the use of Gamma-clone Anti-$\bar{k}$ (Monoclonal) (IgG) must be flushed with large quantities of water to avoid accumulation of potentially explosive compounds in laboratory plumbing.

Handle and dispose of reagent as potentially infectious.

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. Samples drawn into EDTA, ACD, CPD, CP2D and CPDA-1, as well as red blood cells that have been stored in the additive solutions AS-1, AS-3 and AS-5 can be used for testing. The specimen should be tested as soon as possible after collection. If delay in testing should occur, the specimen must be stored at 1°C to 10°C. Bacterial contamination of the specimen may cause false test results. Blood drawn into EDTA should not be stored for longer than ten days. 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:

Gamma-clone Anti-$\bar{k}$ (Monoclonal) (IgG)

Materials Required But Not Provided:

1. Test tubes (12x75 mm or 10x75 mm)
2. Pipettes
3. Isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline pH 6.5-7.5
4. 37°C waterbath or incubator*
5. Timer*
6. Centrifuge*
7. Automated test
8. tube washing device for use with antiglobulin test* (optional)
9. An optical aid such as a hand lens or concave mirror
10. Anti-Human Globulin containing anti-IgG
11. IgG-sensitized red blood cells, such as Checkcell® or Checkcell (Weak)
12. Red blood cells of known Kell phenotypes for use as controls.

*It is the users responsibility to validate an accessory device (either listed or otherwise) for its intended use. Validation results should be maintained as part of the laboratory's records for review by regulatory agencies.

Test Method:

1. Place one (1) drop of Gamma-clone Anti-$\bar{k}$ (Monoclonal) (IgG) into a properly labeled test tube.
2. Add one (1) drop of an approximate 2-5% suspension of the red blood cells to be tested to the test tube (from step 1 above). The red blood cells to be tested must previously have been washed at least one time and then resuspended in saline.
3. Mix the test tube contents well by gently shaking the tube and incubate the tube from five (5) to fifteen (15) minutes at 36°C to 38°C.
4. Wash the tube contents a minimum of three (3) times with 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 one (1) or two (2) drops of Gamma-clone® Anti-Human Globulin (Anti-IgG or Anti-IgG,-C3d; Polyspecific) to the washed button of red blood cells, or follow the directions of the Anti-Human Globulin (AHG) manufacturer.
6. Mix the test tube contents well by gently shaking the tube and centrifuge the tube for:
   (a) one (1) minute between 100 and 125 xg, or
   (b) fifteen (15) seconds between 900 and 1,000 xg, or
   (c) a time and speed appropriate to the calibration of the centrifuge.
7. After centrifugation, immediately resuspend the red blood cells by gently shaking the test tube and examine for macroscopic agglutination. Negative reactions may be examined

with an optical aid; however, microscopic reading is not recommended. Record the results.

8. Confirm all negative reactions by adding one (1) drop of IgG-sensitized red blood cells to each negative test tube.
9. Mix the test tube contents well by gently shaking the tube and centrifuge the test tube from step 8 for:
   (a) one (1) minute between 100 and 125 xg, or
   (b) fifteen (15) seconds between 900 and 1,000 xg, or
   (c) a time and speed appropriate to the calibration of the centrifuge.
10. After centrifugation from step 9, immediately re-suspend the red blood cells by gently shaking the test tube and examine for macroscopic agglutination. Record the 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-$\bar{k}$. Immucor Reagent Red Blood Cells are a convenient source of control cells and may be used as supplied.
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 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	-$\bar{k}$	-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_0	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[9].

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[10], which has been observed in some cases of chronic granulomatous disease[11], 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:

1. Factors that may cause false test results include the following:
   a. Bacterial or chemical contamination of blood specimens, reagent and/or supplementary materials.
   b. Improper storage of materials.
   c. Aged or stored blood specimens, which may yield weaker reactions than those obtained with fresh red blood cells.
   d. Too heavy a red blood cell suspension of the specimen.
   e. Improper incubation time or temperature.
   f. Improper centrifugation. Proper centrifugation calibration is particularly important to the proper performance of the test. Excessive centrifugation may lead to difficulty in resuspending the red blood cell button in the tube test leading to a possible false positive result. At the same time, inadequate centrifugation may yield unclear red blood cell button patterns and agglutinates that are too readily dispersed leading to a possible false negative result.
   g. 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.
   h. Deviation from the recommended test procedure such as the omission of test reagents.
2. 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:

Gamma-clone Anti-$\bar{k}$ (Monoclonal) (IgG) meets FDA potency requirements. Each lot is tested by insert methods against a panel of antigen-positive and antigen-negative red blood cells to ensure suitable reactivity and specificity. The specificity of the human monoclonal antibodies secreted by the cell line used to manufacture this Blood Grouping Reagent has been determined by testing with red blood cells of varying phenotypes.

The performance of this product is dependent upon adhering to the package insert recommended methodology.

Performance Characteristics by manual tube method:

Method comparison studies were performed at three (3) external sites and one (1) internal site. Immucor, Inc., as the manufacturer, was the internal site. The external sites were representative of blood collection establishments, hospital-based transfusion services, and/or clinical laboratories. The sites were selected to capture a diverse sample population based on geographic location and facility demographics. Specimens were tested using both the reagent under evaluation and also a comparator reagent. Test results were evaluated for agreement between reagents. Combined results from all sites are summarized in the following table:

Note: Agreement between methods does not indicate which method is correct.

N=1799		Comparator reagent		[redacted]	
		Positive	Negative		
Anti-$\bar{k}$	Positive	1793	0	PPA (Point Estimate)	100.00%
				PPA (95% 1-Sided LCI)	99.83%
	Negative	0	6	NPA (Point Estimate)	100.00%
				NPA (95% 1-Sided LCI)	60.70%*

*The NPA 95% 1-sided LCI is less than 99% due to the low frequency of k-negative samples (N) in the population.

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

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