

Blood Grouping Reagent IH-Card Rh-Phenotype+K C-E-ϕ-e-K-Ctl

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FOR IN VITRO DIAGNOSTIC USE
Gel card for use with the IH-System
MEETS FDA POTENCY REQUIREMENTS
U.S. LICENSE NUMBER: 1845

Product-Identification: 72000

IH-Card Rh-Phenotype+K:

VOL 12 cards per box
VOL 48 cards per box
VOL 288 cards per box

REF 813 200 100
REF 813 201 100
REF 813 202 100

INTENDED USE

IH-Card Rh-Phenotype+K is intended for the detection of C (RH2), E (RH3), ϕ (RH4), e (RH5), and K (KEL1) antigens on human red blood cells using the IH-System.

SUMMARY

Landsteiner and Wiener first described the Rhesus blood group system in 1940. More than 50 antigens belong to the Rhesus blood group system. The antigens C (RH2), E (RH3), ϕ (RH4), e (RH5) and D (RH1) are the principle antigens of the Rh system. Although many other antigens have been identified, the antibodies associated with these five antigens are responsible for the majority of hemolytic transfusion reactions and cases of Hemolytic Disease of the Newborn associated with the Rh system.

The K (KEL1) antigen is the most important one in the Kell system from a clinical perspective as it is highly immunogenic. Consequently it is a frequently encountered antibody and is associated with transfusion reactions and cases of Hemolytic Disease of the Newborn.

The IH-Card Rh-Phenotype+K can be used for the detection of the C, E, ϕ, e and K antigens on human red blood cells.

PRINCIPLES OF THE TEST

The test combines the principles of hemagglutination and gel filtration for detection of blood group antigen-antibody reactions.

The test sample (red blood cell suspension) is distributed into the microtubes containing the appropriate reagent(s) and centrifuged. Non-agglutinated red blood cells are collected at the bottom of the microtube while the agglutinates are dispersed throughout the length of the gel, depending upon their size. Their position in the gel determines the intensity of the reaction. For the description of the reaction intensity, please refer to the Reaction Grading Guide in the Interpretation of Results section.

REAGENTS

IVD

OBSERVABLE INDICATIONS

Bubbles trapped in the gel, drying of the gel, artifacts, or open or damaged seals may indicate product alteration.

NOTE: INSPECT THE CONDITION OF THE CARDS BEFORE USE (SEE PRECAUTIONS).

IH-Card Rh-Phenotype+K consists of six microtubes containing Anti-C, Anti-E, Anti-ϕ, Anti-e, Anti-K and Control (Ctl). This reagent contains bovine albumin.

Reagent	Source	Antibody Class	Cell lines	Manufacturer
Anti-C	Human Monoclonal	IgM	MS24	Millipore (UK) Limited
Anti-E	Human Monoclonal	IgM	DEM-1	Alba Bioscience Limited
Anti-ϕ	Human Monoclonal	IgM	MS33	Millipore (UK) Limited
Anti-e	Human Monoclonal	IgM	MS16/MS21/MS63	Millipore (UK) Limited
Anti-K	Human Monoclonal	IgM	MS56	Millipore (UK) Limited
Ctl	Gel containing PVP diluent + preservative	-	-	Bio-Rad

Preservative: Sodium Azide (0.1%)

The bovine albumin used for the production of this reagent is purchased from BSE-free sources.

Each card contains six microtubes.

STORAGE REQUIREMENTS

- Store at 18 to 25 °C.
- Do not use beyond expiry on the label, which is expressed as YYYY-MM-DD (Year-Month-Day).
- Store in an upright position.
- Do not freeze or expose cards to excessive heat.
- Do not store near any heat, air conditioning sources or ventilation outlets.

PRECAUTIONS

- All IH-System reagents and test samples must be brought to room temperature (18 to 25 °C) prior to use.
- Do not use cards showing signs of drying.
- Do not use cards with bubbles.
- Do not use cards with damaged foil strips
- Use reagents as furnished.
- Once the IH-Card has been used for testing, it may contain infectious material and should therefore be handled and disposed of as biohazardous waste in accordance with local, state and national regulations.
- Warning: Contains sodium azide, which may react with lead or copper plumbing to form explosive azides. If discarded in the sink, flush with large amounts of water to prevent the buildup of explosive metal azides.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient or donor is required prior to specimen collection. Blood samples should be collected following general blood sampling guidelines.

Fresh blood samples collected in anticoagulant are acceptable. Samples should be tested as soon as possible post collection. If testing is delayed, EDTA samples may be stored at 2 to 8 °C for up to five (5) days or donor blood collected in CPD or CP2D may be tested up to the expiration date of the unit when stored at 1 to 8 °C. Donor blood stored in additive solutions AS-1 or AS-3 may be tested up to thirty (30) days post collection when stored at 1 to 8 °C. Do not use grossly hemolyzed, lipemic or icteric samples.

A distinct separation of red blood cells and plasma is recommended for optimal results. This can be achieved through centrifugation at 10 minutes at 2000g or at a time and speed that consistently produces a distinct cell/plasma interface. Donor segments do not require centrifugation.

TEST PROCEDURE FOR AUTOMATED SYSTEMS

Material provided

- IH-Card Rh-Phenotype+K

Materials required but not provided

- IH-LISS Rack

- IH-1000

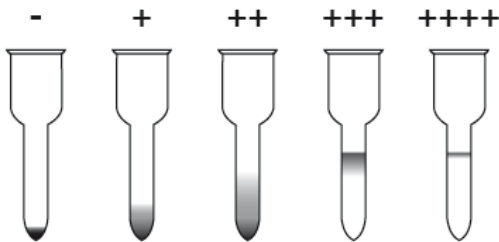
Method

Please refer to the IH-1000 User Manual NA for testing and reagent handling instructions.

INTERPRETATION OF RESULTS

For automated systems

Below is a description of the various reaction grades and how the software uses that well reaction to determine the result interpretation.



Well Reaction Grade	Result Interpretation	Reaction Description
-	Negative	A compact, pellet of RBCs* with a smooth surface at the bottom of the well with no visible agglutination.

+/-	Blood Grouping, Antisera, and Phenotyping including Anti-D Blend, = Not interpretable For Reverse (serum) ABO Testing = Positive Direct Antiglobulin Test, Antibody Detection, Autocontrol = Positive Antibody Identification= no overall result interpretation, only well result shown as +/- For Crossmatching = Incompatible	A pellet of RBCs at the bottom of the well with a very few agglutinated RBCs visible above the pellet or an irregular pellet.
1+	For Blood Grouping, Antisera and Phenotyping including Anti-D Blend = Not interpretable For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification= no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	A pellet of RBCs at the bottom of the well with agglutinated RBCs visible in the lower half of the gel column.
2+	For Blood Grouping, Antisera and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification= no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	Agglutinated RBCs distributed throughout the entire length of the gel column, with no line of RBCs on the top of the well.
3+	For Blood Grouping, Antisera and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification= no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	Most agglutinated RBCs concentrated at the top of the gel or upper half of the gel column.
4+	For Blood Grouping, Antisera and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification= no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	Agglutinated RBCs concentrated as a line on the top of the gel column with a few agglutinated RBCs just underneath the gel surface.
Mixed Field (DP)	Blood Grouping, Antisera, and Phenotyping including Anti-D Blend, = Not interpretable For Reverse (serum) ABO Testing = Positive Direct Antiglobulin Test, Antibody Detection, Autocontrol = Positive Antibody Identification= no overall result interpretation, only well result shown as DP For Crossmatching = Incompatible	Agglutinated RBCs as a line at the top of the gel or dispersed in upper part of the gel and non-agglutinated RBCs forming a pellet at the bottom of the well. The instrument interpretation software displays "DP" (double population) for a mixed field result.
?	For Blood Grouping including Reverse ABO Testing, Antisera, and Phenotyping including Anti-D Blend, Antibody Detection and Identification, Direct Antiglobulin Testing = Not interpretable For Crossmatching = Incompatible	Ambiguous result.

* RBCs = Red Blood Cells

- The control (Ctl) should be negative for the antigen tests on this card to be considered valid.

- This product does not contain ingredients that enhance spontaneous agglutination of immunoglobulin-coated red blood cells, but a false positive test result may still occur due to strong cold autoagglutinins or to a protein imbalance causing the formation of rouleaux. In such cases, similar phenomena would be likely to occur in tests with all the IH System Monoclonal Blood Grouping Reagents. If the control test is positive, laboratories are advised to consult their approved site specific procedures. The test cells can be washed several times in warm saline and retested.¹ If the control test again gives a positive reaction, a valid interpretation of the results obtained cannot be made. Additional testing will be necessary to resolve the false positive reaction according to site-specific procedures.

- Caution must be taken in interpreting a reaction as a mixed field. Additional patient history and testing may be necessary for resolution. Not all mixed field populations have a sufficient minor population to be detected.

Expected reactions with the reagents are shown in the following table:

Reagent	Well Reaction	Interpretation
Anti-C	positive	C+
	negative	C-
Anti- ϕ	positive	ϕ +
	negative	ϕ -
Anti-E	positive	E+
	negative	E-
Anti-e	positive	e+
	negative	e-
Anti-K	positive	K+
	negative	K-

QUALITY CONTROL

On each day of use, the reactivity of all Blood Grouping Reagents should be confirmed by testing with known positive and negative samples. The Blood Grouping Reagents contained on this card could be controlled by testing rr ($\phi\phi ee$), K(-) and R1R2 (DC ϕ Ee), K(+) samples (heterozygous when available). Other combinations of samples are possible as long as there is a positive and negative control for each reagent (this does not apply to the control reagent). Each reagent is satisfactory for use if positive and negative samples react as expected. For additional information, please consult the IH-1000 User Manual NA and the IH-COM User Manual NA, Quality Control Sections

LIMITATIONS

Erroneous and abnormal results may be caused by:

- Bacterial or chemical contamination of the blood specimens, reagents, supplementary materials and/ or equipment.
- Patient medication or disease yielding a cross-reaction.
- A red blood cell concentration or suspension medium different from that recommended.
- Incomplete resuspension of the red blood cells.
- Sample hemolysis prior to testing.
- Contamination between microtubes through pipetting errors.
- Use of procedure other than the one described above.
- Grossly icteric blood samples, blood samples with abnormally high concentrations of protein or blood samples from patients who have received plasma expanders of high molecular weight may give false positive results.
- Fibrin, clots, particulates or other artifacts may cause some red blood cells to be trapped at the top of the gel and may cause an anomalous result.
- A weak reaction is not an expected result for antigen typing and may be indicative of a false positive or weak/partial expression of the antigen. Further investigations may be warranted per site specific procedures.
- The performance characteristics of these reagents have not been established with chemically modified, frozen/thawed or enzyme treated red blood cells.

Please refer to the IH-1000 User Manual NA for instrument specific assay limitations.

SPECIFIC PERFORMANCE CHARACTERISTICS

The final release testing is performed according to the product specific Standard Operating Procedures. As part of the lot release process, each lot of Bio-Rad Blood Grouping Reagents is tested against antigen positive and negative samples to ensure suitable reactivity and specificity.

Performance characteristics on the IH-1000 Analyzer

Testing to determine the performance characteristics of the Bio-Rad IH Blood Grouping Reagents Anti-C, Anti- ϕ , Anti-E, Anti-e and Anti-K was performed at four different US clinical sites and included patient and donor samples. The positive and negative percent agreements were calculated for the Bio-Rad IH Blood Grouping Reagents in comparison to the FDA-licensed reference reagents. Microtube results for a given reagent were combined across applicable IH-Cards.

Results of the positive percent agreement and negative percent agreement, with the one-sided Exact 95% Lower Confidence Limit (LCL) are listed in the data table below. Note: See the IH-1000 User Manual NA and IH-COM User Manual NA for more information on verification of results.

Test	Results from Clinical Trials			
	Negative Agreement		Positive Agreement	
	N	Point Estimate (one-sided Exact 95% LCL)	N	Point Estimate (one-sided Exact 95% LCL)
Anti-C	498	99.40% (98.45%)	1,010	100% (99.70%)
Anti- ϕ	302	99.67% (98.44%)	1,207	100% (99.75%)
Anti-E	1,078	99.72% (99.28%)	431	100% (99.31%)
Anti-e	94	97.87% (93.45%)	1,470	100% (99.80%)
Anti-K	1,383	99.93% (99.66%)	180	100% (98.35%)










Agreement between the methods does not imply which method obtained the correct result. The above results do not reflect any discrepancy resolution between the methods.

Reproducibility was evaluated at two external sites and one internal site by testing a reproducibility panel according to the following scheme: one lot of reagent x 3 sites x 1 operator x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days using the **IH-1000** Analyzer. Reproducibility was demonstrated for the Blood Grouping Reagents Anti-C, Anti- ϕ , Anti-E, Anti-e and Anti-K within runs, between runs and between sites.

A precision study was conducted internally using three reagent lots x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days using the **IH-1000** Analyzer. Precision was demonstrated with all three lots of Blood Grouping Reagents Anti-C, Anti- ϕ , Anti-E, Anti-e and Anti-K.

For technical support or further product information, contact Bio-Rad Laboratories, Inc. at 800-224-6723.

GLOSSARY OF SYMBOLS

Symbol	Definition	Symbol	Definition
	Batch code		<i>In vitro</i> diagnostic medical device
	Caution, consult accompanying documents		Consult instructions for use
	Manufacturer		use by (YYYY-MM-DD)
	Contains sufficient quantity for <n> test.		Catalog number
	Temperature limitation	VOL	Volume

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