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Instructions for Use

Rev. 001/12-2020

Description	REF	
Ant Kell Coombs reactive 10ml **Anti Kell** Coombs reactive 5ml	Kel-coom-0010 Kel-coom-0005	CE 0483
Anti cellano Coombs reactive 2ml **Anti cellano** Coombs reactive 5ml	k-cell-coom-0002 k-cell-coom-0005	CE
Anti Kp^a Coombs creactive 2ml	Kpa-coom-0002	
Anti Kp^b Coombs reactive 2ml	Kpb-coom-0002	

IN VITRO DIAGNOSTIC MEDICAL DEVICE

SUMMARY

The K, k, Kp^a and Kp^b antigens were reported in 1946, 1949, 1957 and 1958, respectively. The antigens of the Kell system are already fully developed at birth, and the K antigen is highly immunogenic. All antigens of the Kell system can cause transfusion reactions and are associated with haemolytic disease of the foetus and newborn (HDFN).

Distribution of antigens in Caucasians in %:

Anti K	Anti k	Anti Kp^a	Anti Kp^b	Phenotype	Caucasians
+	0	N.A.	N.A.	K+k-	0.2
+	+	N.A.	N.A.	K+k+	8.8
0	+	N.A.	N.A.	K-k+	91.0
N.A.	N.A.	+	0	Kp(a+b-)	Rare
N.A.	N.A.	+	+	Kp(a+b+)	2.3
N.A.	N.A.	0	+	Kp(a-b+)	97.7
0	0	0	0	K_0	Extremely rarely

Table 1: Frequency of antigens

INTENDED USE

Anti Kell Coombs reactive, Anti cellano Coombs reactive, AntiKp^a Coombs reactive and Anti Kp^b Coombs reactive are designed for the specific and qualitative detection, by means of the indirect antiglobulin test (IAT), of erythrocytes that carry the corresponding antigen and are suitable only for use by the tube technique.
A specific antigen-antibody-reaction leads to agglutination of the blood if the corresponding antigen is present on the erythrocytes. Lack of agglutination of the red cells demonstrates – taking into account the limitations of the test methods, see below – the absence of the corresponding antigen.

PRODUCT INFORMATION

The polyclonal test sera of human origin are produced from select source material that was tested for the absence of HBsAg, HCV and HIV. Only products unambiguously classified as negative are used for reagent production.
All bovine serum albumin is sourced exclusively from US cattle stocks that tested negative for BSE (certified by the US Animal and Plant Health Inspection Service).
Preservative: < 0.1% sodium azide (final concentration).
The LOT number and expiry date are detailed on the tube label.

STORAGE

Store the test reagents at 2°C-8°C until the expiry date detailed on the product label. After opening a product for the first time, tightly close again and store at 2°C-8°C.

SPECIMEN COLLECTION AND PREPARATION

Blood specimens should be collected in EDTA or citrate tubes in accordance with standard medical procedures. The specimen should be tested as soon as possible following collection. If a delay in testing should occur, store the specimen at 2°C-8°C. Specimens displaying haemolysis or microbial contamination should not be tested with this reagent as this may result in false positive or false negative results.
All blood samples should be washed twice with a 0.9% NaCl solution before being tested by the tube technique.

WARNINGS AND PRECAUTIONS

1. These reagents are intended for *in vitro* diagnostic laboratory use only.
2. These reagents are designed for use by authorized operators trained in serological techniques.
3. These reagents are not intended for self-application.
4. Do not use these reagents past the expiration date.
5. Discard the contents of damaged vials.
6. The test sera contain < 0.1% sodium azide.
7. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
8. The test sera have been filtered through a 0.2µm capsule to reduce the bio-burden.
9. Once a vial has been opened the contents should remain viable up until the expiry date. Discard the contents if turbidity or contamination occur after opening.
10. CE-Immundiagnostika GmbH cannot guarantee that products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.

DISPOSAL OF REAGENT AND DEALING WITH SPILLAGES

For information on disposal of the reagent and decontamination of a spillage site, see the Material Safety Data Sheet, available on request from CE-Immundiagnostika GmbH.

CONTROLS AND ADVICE

1. Positive and negative control erythrocytes shall be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected reactions.
2. Since these reagents do not contain macromolecular potentiators, it is very unlikely that false positive or false negative reactions are caused with IgG coated cells.
3. 1 volume is approximately 35-45µl when using the vial dropper provided.
4. The reading and interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagents are in use.
5. These reagents should be used only according to these instructions for use.

REQUIRED MATERIAL AND REAGENTS

- 0.9% NaCl solution
- Bovine albumin 22% (BSA)
- Anti-human globulin (AHG)
- Glass test tubes
- Test tube holder
- Water bath
- Test tube centrifuge
- Positive and negative control erythrocytes

- Chronometer

RECOMMENDED TECHNIQUES

A. TUBE TECHNIQUE

1. Wash blood twice and prepare a 2-4% suspension of erythrocytes in a 0.9% NaCl solution.
2. Place 1 volume of suspension of test erythrocytes in a labelled test tube.
3. Add 1 volume of Coombs reactive antiserum and mix thoroughly.
   - The addition of 1 volume of BSA 22% will enhance the reaction. While this is not absolutely necessary, it is recommended when weak reactions are obtained.
4. Incubate for 15-30 minutes in a water bath set to 37°C.
5. Wash three times with cold 0.9% saline solution, carefully decant after wash 3.
6. Using the vial dropper, add 2 volumes of AHG and mix thoroughly.
7. Centrifuge 1 minute at 400g (at 1,500 rcf or for a suitable alternative time and force).
8. Read the result immediately: Gently agitate the tube to dislodge the erythrocyte pellet from the bottom of the test tube and read macroscopically for agglutination; record result. Positive and negative control erythrocytes shall be tested in parallel with each batch of tests.

INTERPRETATION OF TEST RESULTS

1. **Positive:** Agglutination of the test erythrocytes indicates, within accepted limitations of test procedure (see below), the presence of the K antigen on the test erythrocytes.
2. **Negative:** No agglutination of the test erythrocytes indicates, within accepted limitations of test procedure (see below), the absence of the K antigen on the test erythrocytes.

LIMITATIONS

1. Stored blood may give weaker reactions than fresh blood.
2. False positive or false negative results may also occur due to:
   • Contamination of test materials
   • Improper storage, erythrocyte concentration, incubation time or temperature
   • Improper or excessive centrifugation
   • Deviation from the recommended techniques
5. Specimens from patients suffering from certain disorders may show false positive/false negative reactions. Cord blood specimens contaminated with Wharton's jelly may show false positive reactions.
6. Erythrocytes that give a positive reaction with the direct antiglobulin test cannot be typed by an indirect antiglobulin test.
7. Antibodies to Di^a, Do^a, Yt^a, Co^b, Wr^a, Bg^a and V^w may be expected to show during a routine test. The detection of these antigens depends on the available test cells.
8. **Always use two different test reagents for the determination of K, k, Kp^a and Kp^b antigens in accordance with the Haemotherapy Guideline, 4.4.8, 2017.**

STABILITY OF THE REACTIONS

1. Read all tube tests straight after centrifugation.
2. Tests must be considered invalid if they have been performed at temperatures other than those recommended.

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance of Anti K Coombs reactive is assessed in accordance with the Common Technical Specifications (CTS: EU Commission Decision of 03/02/2009).

1. The antisera have been tested using all the recommended procedures.
2. Each LOT of Coombs reactive test serum is tested in accordance with the requirements of the Common Technical Specifications by *in vitro* diagnostic medical devices and meets the requirements.
3. Specificity of Coombs reactive antibodies is demonstrated using a panel of antigen-negative erythrocytes.
4. The quality control was performed using erythrocytes that had been washed twice with 0.9% saline solution.
5. Tests with more than 1,000 specimens with a sensitivity and specificity of > 99%.

DISCLAIMER

1. The user is responsible for the performance of the reagents by any method other than those recommended.
2. Any deviations from the Recommended Techniques should be validated prior to use.

BIBLIOGRAPHY

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INDEX OF SYMBOLS

LOT	Lot number	IVD	For *in vitro* diagnostic use only
REF	Catalogue number		Store between (°C)
	Expiry date		Manufacturer
	Consult instructions for use (insert)		

CATALOGUE NUMBERS

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Anti Kell Coombs reactive
Anti cellano Coombs reactive
Anti Kpa Coombs reactive
Anti Kpb Coombs reactive

REF	Quantity
Kel-coom-0010-01	1 x 10ml
Kel-coom-0010-05	5 x 10ml
Kel-coom-0010-10	10 x 10ml
Kel-coom-0010-20	20 x 10ml
Kel-coom-0010-50	50 x 10ml
Kel-coom-0005-01	1 x 5ml
Kel-coom-0005-05	5 x 5ml
Kel-coom-0005-10	10 x 5ml
Kel-coom-0005-20	20 x 5ml
Kel-coom-0005-50	50 x 5ml
k-cell-coom-0002-01	1 x 2ml
k-cell-coom-0002-05	5 x 2ml
k-cell-coom-0002-10	10 x 2ml
k-cell-coom-0002-20	20 x 2ml
k-cell-coom-0002-50	50 x 2ml
k-cell-coom-0005-01	1 x 5ml
k-cell-coom-0005-05	5 x 5ml
k-cell-coom-0005-10	10 x 5ml
k-cell-coom-0005-20	20 x 5ml
k-cell-coom-0005-50	50 x 5ml
Kpa-coom-0002-01	1 x 2ml
Kpa-coom-0002-05	5 x 2ml
Kpa-coom-0002-10	10 x 2ml
Kpa-coom-0002-20	20 x 2ml
Kpa-coom-0002-50	50 x 2ml
Kpb-coom-0002-01	1 x 2ml
Kpb-coom-0002-05	5 x 2ml
Kpb-coom-0002-10	10 x 2ml
Kpb-coom-0002-20	20 x 2ml
Kpb-coom-0002-50	50 x 2ml