DIALAB Produktion und Vertrieb von chemisch – technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
IZ NOE-Sued, Hondastrasse, Objekt M55 A – 2351 Wiener Neudorf, Austria,
Phone: ++43 (0) 2236 660910-0, Fax: ++43 (0) 2236 660910-30, e-mail: office@dialab.at

Anti-k, monoclonal

HUMAN BLOOD GROUPING REAGENTS

For Indirect Antiglobulin Techniques

REF Cont.

B18908 **1x 2 mL** **Anti-k, monoclonal**

For professional in vitro diagnostic use only.

SUMMARY

The k (Cellano) antigens were reported in 1949. Anti-k has been implicated in Haemolytic Transfusion Reactions and Haemolytic Disease of the Newborn.

Anti-K	Anti-k	Phenotype	Percentage
+	0	**K+k-**	0.2
+	+	**K+k+**	8.8
0	+	**K-k+**	91.0

PRINCIPLE

The reagent will cause indirect agglutination (clumping) of test red cells, that carries the corresponding specific antigen, in the antiglobulin phase of testing. No agglutination generally indicates the absence of the corresponding specific antigen (see Limitations).

REAGENTS

This Monoclonal IgG blood grouping reagent contains human monoclonal antibodies diluted in a phosphate buffer containing sodium chloride and bovine albumin. The reagent is supplied at optimal dilution for use with all the recommended techniques stated below without the need for further dilution or addition. For lot reference number and expiry date see Vial Label.

Product	Cell Line/Clone
Anti-k (Cellano)	P3A118OL67

STORAGE

Do not freeze. Reagent vials should be stored at 2 - 8ºC on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity. The reagent has undergone transportation stability studies at 37°C and -25°C as described in EN23640:2011.

SAMPLE COLLECTION AND PREPARATION

Blood samples can be collected into EDTA, citrate, CPDA anticoagulants or as a clotted sample. The samples should be tested as soon as possible following collection. If a delay in testing should occur, store the samples at 2-8°C. Samples displaying gross haemolysis or microbial contamination should not be used for testing. Blood samples showing evidence of lysis may give unreliable results. It is preferable to wash all blood samples with PBS or Isotonic saline before being tested.

CONTROLS AND ADVICE

1. It is recommended a positive control (ideally heterozygous cells) and a negative control be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. The antiglobulin techniques can only be considered valid if all negative tests react positively with IgG sensitised red cells.
3. The reagents contain macromolecular potentiators which may cause false positive reactions with IgG sensitised cells, it is recommended that patient's cells are tested with patient's plasma to test for false positive reactions.
4. In the **Tube Technique** one volume is approximately 50 µL when using the vial dropper provided.
5. The use of the reagents and the 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.
6. User must determine suitability of the reagents for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- Anti-Human Globulin (e.g. DIALAB Anti-HG polyspecific/monoclonal, REF: B05181) or anti-IgG
- Coombs cell washer
- Bio-Rad / DiaMed ID-Cards (LISS/Coombs or Coombs Anti-IgG).
- Bio-Rad / DiaMed ID-Centrifuge
- Bio-Rad / DiaMed ID-CellStab or ID-Diluent 2.
- Bio-Rad / DiaMed ID-Incubator equilibrated to 37ºC ± 2ºC
- Glass test tubes (10 x 75 mm or 12 x 75 mm)
- IgG sensitised red cells
- Ortho BioVue System Cassettes (AHG/Coombs)
- Ortho BioVue System Centrifuge
- Ortho BioVue System Heat Block equilibrated to 37°C ± 2ºC
- Ortho 0.8% Red Cell Diluent
- PBS solution (pH 6.8 – 7.2) or Isotonic saline solution (pH 6.5 – 7.5)
- Positive (ideally heterozygous) and negative control red cells
- Volumetric pipettes
- Water bath or dry heat incubator equilibrated to 37ºC ± 2ºC

RECOMMENDED TECHNIQUES

A. Indirect Antiglobulin Technique (IAT)

1. Prepare a 2-3% suspension of washed test red cells in PBS or Isotonic Saline.
2. Place in a labelled test tube: 1 volume of DIALAB Anti-k, monoclonal reagent and 1 volume of test red cell suspension.
3. Mix thoroughly and incubate at 37ºC for 15 minutes.
4. Wash test red cells 4 times with PBS or Isotonic saline, taking care to decant saline between washes and resuspend each red cell button after each wash. Completely decant saline after last wash.
5. Add 2 volumes of anti-human globulin or anti-IgG to each dry cell button.
6. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
7. Gently resuspend red cell button and read macroscopically for agglutination
8. Confirm validity of all negative reactions with IgG sensitised red cells.

B. Bio-Rad/DiaMed-ID Micro Typing Technique

1. Prepare a 0.8% suspension of washed test red cells in ID-CellStab or ID-Diluent 2.
2. Remove aluminium foil from as many microtubes as needed on either LISS/Coombs or Coombs Anti-IgG ID cards.
3. Place in appropriate microtube: 50 µL of test red cell suspension and 25 µL of DIALAB Anti-k, monoclonal reagent.
4. Incubate the LISS/Coombs ID-Card(s) for 15 minutes at 37ºC.
5. Centrifuge the LISS/Coombs ID-Card(s) in a Bio-Rad/Diamed ID-Card centrifuge.
6. Read macroscopically for agglutination.

C. Ortho BioVue Typing Technique

1. Prepare a 0.8% suspension of washed test red cells in 0.8% Ortho Red Cell Diluent.
2. Remove aluminium foil from as many reaction chambers as needed on either AHG Polyspecific or AHG Anti-IgG cassettes.
3. Place in appropriate reaction chamber: 50 µL of test red cell suspension and 40 µL of DIALAB Anti-k, monoclonal reagent.
4. Incubate cassette(s) 15 minutes at 37ºC.
5. Centrifuge cassette(s) in an Ortho BioVue System Centrifuge.
6. Read macroscopically for agglutination.

INTERPRETATION OF TEST RESULTS

1. **Positive:** Agglutination of the test red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the k- antigen on the test red cells.
2. **Negative:** No agglutination of the test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of the k-antigen on the test red cells.

STABILITY OF THE REACTIONS

1. Washing steps should be completed without interruption and tests centrifuged and read immediately after addition of the reagent. Delays may result in dissociation of antigen-antibody complexes, causing false negative or weak positive results.
2. Caution should be exercised in the interpretation of results of tests at temperatures other than those **recommended**.

LIMITATIONS

1. Red cells that have a positive DAT due to a coating of IgG cannot be typed by the **Indirect Antiglobulin Technique.**
2. Suppressed or diminished expression of certain blood group antigens may conversely give rise to false negative reactions and so caution should always be exercised when assigning genotypes on the basis of test results.
3. False positive or false negative results may also occur due to:
   - Contamination of test materials
   - Improper storage, cell concentration, incubation time or temperature
   - Improper or excessive centrifugation
   - Deviation from the recommended techniques

PERFORMANCE CHARACTERISTICS

1. The reagents have been characterised by the procedures mentioned in the **Recommended Techniques**.
2. Prior to release, each lot of DIALAB Anti-k, monoclonal reagent is tested by the **Recommended Techniques** against a panel of antigen-positive red cells to ensure suitable reactivity.
3. Specificity of source monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
4. The Quality Control of the reagents was performed using red cells that had been washed twice with PBS or Isotonic saline prior to use.
5. The reagents comply with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services

DISCLAIMER

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

PRECAUTIONS

1. The reagents are intended for in vitro diagnostic use only.
2. If a reagent vial is cracked or leaking, discard the contents immediately.
3. Do not use the reagents past the expiration date (see **Vial Label**).
4. Do not use the reagents if a precipitate is present.
5. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
6. The reagents have been filtered through a 0.2 µm capsule to reduce the bioburden. Once a vial has been opened the contents should remain viable up until the expiry date as long as there is no marked turbidity, which can indicate reagent deterioration or contamination.

7. The reagents contain 0.1% sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive metal azides. On disposal, flush away with large volumes of water.
8. Materials used to produce the reagents were tested at source and found to be negative for HIV 1+2 and HCV antibodies and HBsAg using approved microbiological tests.
9. No known tests can 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 reagents and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

BIBLIOGRAPHY

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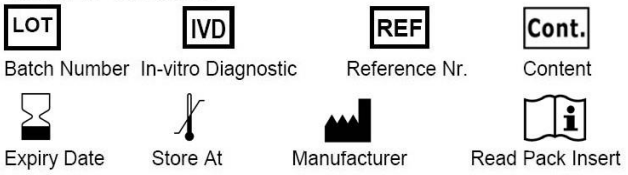
4. Issitt PD. Applied Blood Group Serology, 3rd Edition. Montgomery Scientific, Miami 1985; Chapter 6
5. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.
6. British Committee for Standards in Haematology, Blood Transfusion Task Force. Recommendations for evaluation, validation and implementation of new techniques for blood grouping, antibody screening and cross matching. Transfusion Medicine, 1995, 5, 145-150.

TABLE OF SYMBOLS



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