Anti A, Anti B, Anti A+B

RUO

CONTENTS			
REF	3420010	Anti A	10 mL
	3430010	Anti B	10 mL
	3450010	Anti A+B	10 mL
For *in vitro* diagnostic use only			

ABO

Anti A, anti B, anti A+B monoclonal

Qualitative determination of the A and/or B antigens on human red blood cells.

SLIDE AND TUBE TESTS

PRINCIPLE

ABO grouping is determined by testing unknown red cells against known anti A, anti B and anti A+B antibodies.
The presence or absence of agglutination of the red cells indicates the presence or absence the corresponding antigen.
Blood grouping reagents made with monoclonal antibodies have the added advantage of constant identity and absolute reproducibility of their specificity.

Forward Group			Reverse Group				ABO	%
A	B	A,B	A_1	A_2	B	O	Phenotype	Caucasians
+	0	+	0	0	+	0	**A**	42
0	+	+	+	+	0	0	**B**	10
0	0	0	+	+	+	0	**O**	44
+	+	+	0	0	0	0	**AB**	4

REAGENTS COMPOSITION

Mouse monoclonal IgM antibody.

Anti A — Cell line 9113D10. Phosphate buffer. Sodium azide <0.1%. Blue colour. Dye used: Patent Blue

Anti B — Cell line 9621A8. Phosphate buffer. Sodium azide <0.1%. Yellow colour. Dye used: Tartrazine.

Anti A+B — Cell line 152D12+9113D10. Phosphate buffer. Sodium azide <0.1%. Colourless.

Precautions: It is advisable handling reagents and samples with the proper precautions.
Wear gloves and protective clothing.

Warning: The reagents in this kit contain sodium azide. Do not allow to contact with skin or mucous membranes.

STORAGE AND STABILITY

1. The reagents will remain stable until the expiration date printed on the label, when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not use the reagents after the expiration date.
2. Do not freeze or expose to elevated temperatures. Prolonged storage outside the recommended temperature range may result in accelerated loss of reagent reactivity.
3. This product should be clear. Turbidity may indicate microbial contamination. Do not use the reagents if a precipitate is present.
4. If a vial is cracked or leaking, discard the contents immediately.

REAGENT PREPARATION

The reagents are ready to use.

SAMPLES

The blood samples can be collected with or without anticoagulant. They must be tested as soon as possible.
Samples collected into EDTA or heparin should be tested within 48 hours.
Blood collected into ACD, CPD, CPDA-1 may be typed up to 35 days from the date of withdrawal.
Store at 2º-8ºC.

MATERIALS REQUIRED

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Pasteur pipettes.
- Centrifuge Sero-fuge or similar.
- Glass slides.
- Applicator sticks.

ADDITIONAL REAGENT REQUIRED

- Phosphate Buffered Saline (PBS):
  8.5 to 9.0 g/L NaCl (0.145-0.154 mol/L) pH 7.0±0.2 at 22 ±1ºC.
- Test red cells. Positive (ideally group A_2B) and negative (group O).

PROCEDURE

I. Slide Test

1. Place 1 drop (approx. 40 µL) of resuspended whole blood (approx. 35-40% cell concentration) to be tested on a slide.
2. Add 1 drop (approx. 40 µL) of reagent next to the blood sample.
3. Mix the reagent and cells with an applicator stick, over an area of about 2 cm. in diameter.
4. Rotate gently and continuously the slide test during a 2-minute period.

Reading

Examine macroscopically for agglutination.
Negative reaction:
No visible agglutination after 2 minutes.
Positive reaction:
Positive red cells agglutinate in a few seconds.
However it is recommended to make a final reading at the end of 2 minutes.

II. Tube test

1. Prepare a 2-3% suspension of red cells washed (twice with PBS) in PBS.
2. Place 1 drop (approx. 40 µL) of reagent into an appropriate test tube.
3. Add 1 drop (approx. 40 µL) of washed red cells.

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4. Mix well, incubate at 18-25ºC for at least 1 minute and centrifuge for 20 sec. at 1000 r.c.f. or for a suitable alternative time and force.
5. **Reading**
   Gently resuspend each cell button and read macroscopically for agglutination.
   Negative reaction:
   A smooth homogeneous suspension indicates a negative reaction.
   Positive reaction:
   Agglutination of the red cells indicates a positive reaction.
   Any tube showing a negative or a questionable test result should be incubated for 15 minutes at 18-25ºC.
   Following incubation, repeat steps 4 and 5.

Interpretation

1. *Positive reaction*: Agglutination of the test red cells constitutes a positive test result and within accepted limitations of test procedure, indicating the presence of the appropriate ABO antigen on the test red cells.
2. *Negative reaction*: 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 appropriate ABO antigen on the test red cells.

Discrepancies:

- If the results obtained with reverse group do not correlate with forward group, further investigation is required.
- Test results of cells that are agglutinated using the reagent negative control shall be excluded, as the agglutination is most probably caused by the effect of the macromolecular potentiators in the reagent on sensitized cells.

QUALITY CONTROLS AND ADVICE

- It is recommended a positive control (ideally group A_2B cells) and a negative control (ideally group O cells) be tested in parallel with each batch of tests.
  Tests must be considered invalid if controls do not show expected results.
- When typing red cells from a patient it is important that a reagent negative control is included since the macromolecular potentiators in the reagent may cause false positive reactions with IgG coated cells.
- Individuals older than six months should have their ABO blood-grouping results confirmed by testing their serum or plasma against known group A_1 and B cells before their ABO blood group can be confirmed.
- Use of reagents and interpretation of results must be carried out by properly trained and qualified personnel in accordance with requirements of the country where the reagents are in use.
- The user must determine the suitability of the reagents for use in other techniques.

ANALYTICAL PERFORMANCE

- The reagents have been characterized by all the procedures mentioned in the PROCEDURE.
- The potency of the reagents is tested for against the following minimum potency reference standards obtained from National Institute of Biological Standards and Controls (NIBSC):
  - Anti-A reference standard 88/722 **And / Or**
  - Anti-B reference standard 88/724
- Linear Chemicals Anti-B does not react with “Acquired-B” red cells.
- Linear Chemicals Monoclonal ABO reagents do not detect cryptantigens such as T, Tn or Cad.

LIMITATIONS OF THE PROCEDURE

- ABO antigens are not fully developed at birth and so weaker reactions may therefore occur with cord or neonatal specimens.
- Blood specimens of weak A or B subgroups may give rise to false negative or weak reactions.
- Stored blood may give weaker reactions than fresh blood
- False positive or false negative results may also occur due to:
  - Contamination of test materials
  - Improper incubation time or temperature
  - Improper or excessive centrifugation
  - Improper storage of test materials or omission of reagents
  - Deviation from the recommended techniques Cord samples contaminated with Wharton’s jelly

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