

Fortress Forward & Reverse Grouping (BLOOD GROUPING GEL CARDS)	
BGGCFRG1-12	BGGCFRG1-24
12 FORTRESS GEL CARDS	24 FORTRESS GEL CARDS
STORE AT 2-8°C	
FOR IN-VITRO DIAGNOSTIC USE ONLY	

INTENDED USE

This test allows the detection of ABO, Rh D, Rh D^{VI} and reverse grouping in gel technique.

INTRODUCTION

Classification of blood groups should be based on both forward and reverse grouping. The ABO blood group system is determined directly by testing the red blood cells with Anti-A and Anti-B reagents. Confirmation of the test results is provided by testing the serum with known group A1 and group B red blood cells. Fortress Forward and Reverse Grouping test allows the detection of ABO, Rh D, Rh D^{VI} and reverse grouping.

PRINCIPLE OF TEST

Fortress Forward and Reverse Grouping test allows the detection of forward and reverse ABO, Rh D, Rh D^{VI} antigen grouping simultaneously on a single gel card. The microtubes contain gel matrix. First five microtubes contain monoclonal antibodies and last three microtubes don't contain antibody in the gel.

The Anti-A, Anti-B, Anti-D and control microtubes are used for ABO and RhD antigen forward grouping. The microtubes without antibodies are used for reverse grouping. The red blood cells will agglutinate in the presence of antibody directed towards the antigen. Agglutinated cells forming a red button on the surface of the gel or agglutinates dispersed in the gel is a positive result and indicates the presence of the corresponding antibody.

KIT CONTENTS

12 or 24 Fortress Forward and Reverse Grouping gel cards.

1 gel card contains 8x1 microtubes

A	B	AB	DVI-	DVI+	Ctl	A1 _{cell}	B _{cell}
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Ready to use. Contains Sodium azide as preservative.

Each microtube of the card contains buffered gel medium with preservative and mixed different reagent.

- microtube A : monoclonal anti-A IgM (clone A-11H5)
- microtube B : monoclonal anti-B IgM (clone B-6F9)
- microtube AB : monoclonal anti-AB (clones LA2/ LB2/ ES15)
- microtube D^{VI}- : Mixture of IgG and IgM antibodies (clone BS 225, LDM3, ESD1)

This anti-D monoclonal reagent detects weak D and partial variants of the D antigen. But D^{VI} variant of the D antigen will not be detected directly with this monoclonal reagent

- microtube D^{VI}+ : monoclonal anti-D IgM (clones LDM1 / ESD1M)

This anti-D monoclonal reagent detects weak D and partial variants of the D antigen, including the D^{VI} variant. The ESD1M antibody will directly detect Rh D^{VI} red cells

- microtube Ctl.: buffered solution without antibodies (control microtube)
- microtube A1 cell: buffered solution without antibodies (reverse ABO group test)
- microtube B cell: buffered solution without antibodies (reverse ABO group test)

MATERIAL REQUIRED BUT NOT PROVIDED

- 10 µl, 50 µl and 1 ml automatic pipettes.
- Disposable pipette tips.
- Test tubes.
- Fortress LISS Diluent
- Centrifuge for Blood Grouping Gel System.
- Red blood cells for reverse group test (A1/B).

STORAGE CONDITIONS AND STABILITY

- Store the gel cards at 2-8 °C in upright position.
- Do not freeze.
- Avoid exposure of the cards to any heat source, direct air-conditioning sources or direct sunlight.
- Do not use reagents over the expiration date.

-All the components of the kit are stable until the expiration date on the label when stored 2-8 °C.

- Do not remove foil seal until ready to use. Once opened, the gel may begin to dry out which could affect test results.

- Use the card within 2 hours after removing the aluminum foil . Otherwise, don't use the card.

- Stability of the opened gel card is 2 hours.

PRECAUTIONS AND WARNINGS

- This reagent kit is for in vitro diagnosis only.
- This reagent kit is for professional use only.
- All human sourced material (monoclonal antibodies) are tested free from HBsAg, Anti-HCV and Anti-HIV. However, all materials of human origin should be considered as potentially infectious and it is recommended that all kit materials and test specimens to be handled using established good laboratory practice.
- All kit components and specimens should be regarded as potential hazards to health. It should be used and discarded according to your own laboratory's safety procedures.

SAMPLE COLLECTION

Fresh red blood cells are preferred for testing . Use the red blood cells collected with anticoagulants for determination of the antigens of the ABO/Rh system . If necessary, samples stored at 2-8 °C can be used up to 48 hours . Red blood cells collected in ACD, CPD, SAGM and PAGGSM can also be used until the expiry date indicated on the label of the bag at 2-8 °C.

Plasma or serum can be used for reverse grouping. Plasma and serum must be cleared . Fibrin residues may interfere with the reaction pattern.

PREPARATION OF BLOOD SAMPLE

5 % red blood cell suspension :

Do not use haemolysed, cloudy or contaminated samples or those containing clots

- 1- Bring Fortress LISS Diluent to reach room temperature before use
- 2- Dispense 0,5 mL of Fortress LISS Diluent into a clean tube.
- 3- Add 50 µL of whole blood or 25 µL of packed cells to the diluent and mix gently.

0.8 % red blood cell suspension :

Do not use haemolysed, cloudy or contaminated samples or those containing clots.

Collect known A1 and B cells. Wash the cells twice with 0.9% saline. Discard the supernatant and prepare 0.8% cell suspension

- 1- Bring Fortress Diluent 1 to reach room temperature before use
- 2- Dispense 1 mL of Fortress Diluent 1 into a clean tube.
- 3- Add 20 µL of whole blood or 10 µL of packed cells to the diluent and mix gently.

Plasma or Serum for reverse grouping:

If necessary, samples stored at 2-8 °C can be used up to 48 hours after their extraction; or frozen samples (from -20 °C to -70 °C) up to the time of performing the test.

TEST PROCEDURE

Allow the test card and cell reagent to reach room temperature before use.

1- Label the appropriate gel card with patient's name or identification number. Remove the aluminium foil carefully.

2- Pipette 10-12 µL (5 %) or 50 µL (0.8 %) of the patient's red cell suspension to the microtubes 1– 6 (A, B, AB, D^{VI}-, D^{VI}+, Ctl).

3- Pipette 50 µl of 0.8% A1 cell suspension to the microtube 7 (A1 cell).

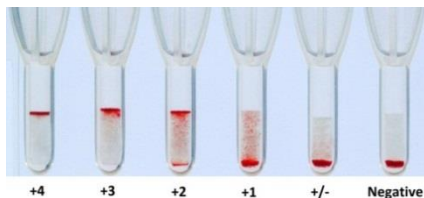
4- Pipette 50 µl of 0.8% B cell suspension to the microtube 8 (B cell).

5- Pipette 50 µL of the patient serum or plasma to both microtubes 7 and 8.

6- Allow the cards to incubate for 10 minutes at room temperature (18– 25 °C).

7- Centrifuge the gel cards for 10 minutes in the gel card centrifuge.

8- Read the results.



Negative	Band of red blood cells at the bottom of the column and no visible agglutinations in the rest of the column.
+/-	Scarce small-sized agglutinations in the lower half of the column
+1	Most agglutinated red blood cells remain in the lower half of the column. A button of cells may also be visible at the bottom of the gel column
+2	Agglutinated red blood cells are observed throughout the length of the column. A small Agglutinated red blood cells are observed throughout the length of the column.
+3	Most agglutinated red blood cells remain in the upper half of the gel column
+4	Agglutinated red blood cells form a band at the top of the gel column

Stability of the results:

It is recommended an immediate reading of the results after centrifuging the cards. Do not leave processed cards in horizontal position. If necessary, a delayed reading can be made up until 24 hours after processing the cards if kept in vertical position, refrigerated (2-8°C) and sealed with parafilm or similar material to avoid evaporation of the supernatant.

Interpretation of the results:

ABO system

Anti A	Anti B	Anti AB	Ctl
Negative	Negative	Negative	Negative
+1 to +4	Negative	+1 to +4	Negative
Negative	+1 to +4	+1 to +4	Negative
+1 to +4	+1 to +4	+1 to +4	Negative

The microtube Ctl must be negative. If it is positive, invalidate the test.

The reactions between +/- and +3 may indicate A or B subgroups and further investigations should be performed

Rh System

D ^{vi} -	D ^{vi} +	
+1 to +4	+1 to +4	RhD Positive
Negative	Negative	RhD Negative
Negative	+1 to +4	D ^{vi} Positive
+1 to +4	Negative	D ^{vi} Negative

The reactions weaker than +2 should be subject to further investigations to distinguish between weak and partial D types as appropriate for the category of sample being tested.

Reverse Group

A1 cell	B cell	ABO Group
+1 to +4	+1 to +4	O
Negative	+1 to +4	A
+1 to +4	Negative	B
Negative	Negative	AB

If questionable reactions are obtained, repeat reverse grouping with 4 red cell reagents (A1, A2, B and O).

LIMITATIONS

- The Aluminum foil on the matrix gel card should be removed carefully and gently.
- Do not use matrix gel cards, which show signs of drying.
- The gel cards which show air bubbles in the gel or drops in the upper part of the microtubes and/or the seal must be centrifuged before use.
- Do not touch the pipet tip inside the gel card. Carryover problem may occur.
- Do not use hemolysed, cloudy or contaminated samples or with clot presence.
- Red blood cells from individuals with A or B variants may present a weak expression of the antigens.

-Abnormal concentrations of serum proteins, the presence of macromolecular solutions in the serum may cause the non-specific agglutination of the red blood cells. It is recommended to wash the red blood cells before performing the test.

- Transfused patients or those subjected to bone marrow transplant may present images of double population.

- Patients with high-potency cold antibodies may coat the red blood cells completely so spontaneous agglutination may occur.

PERFORMANCE CHARACTERISTICS

Diagnostic sensitivity and specificity of ABO/Rh system :

The diagnostic sensitivity and specificity of the antibodies present in Fortress Forward and Reverse Grouping test for the determination of the antigens of the ABO and Rh systems have been studied in a representative number of positive and negative samples. In the study using 3092 blood samples the sensitivity and specificity of ODAK Blood Grouping Gel System was as follows.

	Number of samples	Sensitivity	Specificity
Anti-A	3092	% 100	% 100
Anti-B	3092	% 100	% 100
Anti-D	3092	% 100	% 100

Reverse ABO group

We conducted our own performance study in 240 different samples and obtained same results with other established products of equivalent intended use. Fortress Forward and Reverse Grouping test performance characteristics is suitable for the determination of the reverse group

Precision:

Inter-assay and intra-assay reproducibility tests; no false positive or false negative results were obtained. Variability between agglutination views in positive samples were 1 agglutination rating or less in all assays.

BIBLIOGRAPHY

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Label Symbols

- Batch code
- Expiry date
- Storage temperature
- Consult instructions for use
- In vitro diagnostic medical device
- Product code
- Manufacturer
- Harmful