rBGA

Version 25_EN_RUO, 2026-10

RUO	For research use only
Σ	PU 300 µl: 75 – 150 PU 100 µl: 25 – 50 (CD36 only)
2°C 8°C	Store at 2...8°C
	See package printings

1. Introduction

1.1. Overview

This instructions for use describes the protocol for the imusyn

recombinant blood group antigens (rBGA)

for the inhibition of irregular anti-erythrocytic antibodies.

The detection of antibodies against erythrocyte blood group antigens is a central step in the scientific analysis of pre- and post-transfusion specimens.

However, in some cases (e.g. antibody mixtures, autoantibodies or antibodies against high frequency erythrocyte antigens) a clear identification of the antibodies can be difficult or even impossible[1]. Irrelevant antibodies can mask relevant antibodies and interfere with the analysis. rBGA are able to specifically inhibit anti-erythrocyte antibodies[2].

1.2. Test Principle

rBGA work as inhibiting molecules. The specimen is incubated with rBGAs prior to an indirect antiglobulin test (IAT) to inhibit specific antibodies against the according BGAs. It can then be investigated in the IAT for the presence of other antibodies without any further treatment.

1.3. Statement of Intended Use

For research use only.

To be used by qualified personnel only.

Inhibition of irregular anti-erythrocytic antibodies. rBGAs are for use with gel card systems (Grifols DG Gel, Bio-Rad ID-System, ORTHO BioVue, ORTHO MTS, or Cellbind Screen) or in tube testing.

2. Materials and Equipment

2.1. Components

Recombinant blood group antigens rBGA, 300 µl (100 µl) total volume, concentration: 0.5 mg/ml (0,25 mg/ml). Conserved with 0.1% ProClin® 300



WARNING!

May cause an allergic skin reaction (H317). Harmful to aquatic life with long lasting effects (H412). Wear protective gloves (P280). If skin irritation or rash occurs: Get medical advice/attention (P333+P313). Dispose of contents/container in accordance with local/regional/national/international regulations (P501).

Safety data sheet (SDS) available on imusyn.de/IFU.

2.2. Table of Antigens

REF	Antigen
R_Ch(a)	**CH1**
R_CR1_2	**KN1**, KN3, KN4, KN5, KN8, KN9, **KN11**, KN13
R_CROM	CROM1, CROM2, **CROM5**, CROM6, CROM7, CROM9, CROM10, CROM11, CROM12, CROM13, CROM14, CROM15, CROM16, CROM17, CROM18, CROM19, CROM20
R_Do(a)	**DO1**, DO3, DO4, DO5, DO6, DO7, DO8, DO9, DO10
R_Do(b)	**DO2**, DO3, DO4, DO5, DO6, DO7, DO8, DO9, DO10
R_Fy(a)	**FY1**, FY6
R_Fy(b)	**FY2**, FY6
R_grKba	**KEL1**, **KEL4**, KEL5, KEL6, KEL11, KEL12, KEL13, KEL14, KEL16, KEL18, KEL19, KEL20, KEL22, KEL25, KEL26, KEL27, KEL28, KEL29, KEL30, KEL32, KEL33, KEL34, KEL35, KEL36, KEL37, KEL38, KEL40
R_In(b)	**IN2**, IN3, IN4, IN5, IN6
R_JMH	**JMH1,** JMH2, JMH3, JMH4, JMH5, JMH6, JMH7, JMH8
R_klkba	**KEL2**, **KEL4**, KEL5, KEL6, KEL11, KEL12, KEL13, KEL14, KEL16, KEL18, KEL19, KEL20, KEL22, KEL25, KEL26, KEL27, KEL28, KEL29, KEL30, KEL32, KEL33, KEL34, KEL35, KEL36, KEL37, KEL38, KEL40
R_Lu(a)_2	**LU1**, LU4, LU5, LU6, LU7, LU8, LU12, LU13, LU16, LU17, LU18, LU20, LU21, LU22, LU23, LU24, LU25, LU26, LU27, LU28, LU29, LU30
R_Lu(b)_2	**LU2**, LU4, LU5, LU6, LU7, LU8, LU12, LU13, LU16, LU17, **LU19**, LU20, LU21, LU22, LU23, LU24, LU25, LU26, LU27, LU28, LU29, LU30
R_LW(a)	**LW5,** LW8
R_Rg(a)	**RG1**
R_Sc1	**SC1,** SC3, SC5, SC6, SC7, SC8, SC9
R_Xg(a)	**XG1**
R_YCAD	**KN12**, KN13
R_Yt(a)	**YT1**, YT3, YT4, YT5, YT6
R_CD36	**CD36**
R_B07:02T	**HLA-B*07:02**

Table 1) List of rBGA with corresponding antigens. Serologically tested antigens are in bold.

2.3. Storage and Expiry Date

Store at 2…8°C. The expiry date is given on the label of the immediate container. If the storage conditions are met, the proteins can be used until the expiry date given on the immediate container and the certificate of analysis.



Do not freeze rBGA! The reactivity of frozen or frozen and thawed proteins cannot be guaranteed. The according proteins have to be disposed of immediately!

2.4. Materials and Equipment Supplied by the User

The following materials and equipment have to be provided by the user:

2.4.1. Materials

Material	Supplier
Gel cards: DG Gel Coombs, ID-Card LISS/Coombs, ORTHO BioVue, ORTHO MTS, or Cellbind Screen	Grifols, Bio-Rad, Ortho Diagnostics, Sanquin
Test cell reagents for the according gel card system	Grifols, Bio-Rad, Ortho Diagnostics, Sanquin
Alternatively: materials and test cells for tube testing	Multiple suppliers
Polypropylene reaction tube	Multiple suppliers
PBS Phosphate buffered saline, pH 7.4 or NaCl 0.9% sodium chloride solution	Multiple suppliers
Pipette tips	Multiple suppliers

2.4.2. Equipment

Equipment	Supplier
Gel card centrifuge or work station, matching the gel card system used	Grifols, Bio-Rad, Ortho Diagnostics, Sanquin
Alternatively: centrifuge for tube testing	Multiple suppliers
Incubator, 37°C	Multiple suppliers
Pipettes	Multiple suppliers
Tabletop centrifuge	Multiple suppliers

Note: The reagents and instruments listed above with a specific supplier given have been validated for the use with rBGAs. Reagent and instruments other than those must be validated by the user before use.

imusyn GmbH & Co. KG · Feodor-Lynen-Str. 5 · 30625 Hannover, Germany · Tel: +49 511 563585-0 · Fax: +49 511 563585-229 · www.imusyn.de · info@imusyn.de

3. Preparation and Usage

Contaminations have to be avoided during all steps.

⚠ Only use rBGA that come in undamaged containers! All components have to be disposed of according to local regulations.

3.1. Specimen Preparation

Serum or plasma specimen have to be fresh and neither hemolytic nor lipaemic. Preferably, specimen should be drawn into citrate, EDTA or CPD-A anticoagulant. Particles, aggregates, or fibrin residues have to be removed prior to testing to avoid unspecific reactions. Samples should be stored at 2..8°C for no longer than 48 hrs. Also follow the restrictions for specimens given by the manufacturer of your gel card system.

⚠ Human specimens pose a potential health hazard. Treat the specimens as potentially infectious and take according protective measures as stated in your local guidelines.

3.2. Test Procedure

Pre-incubation of rBGA and specimen in a test tube

Test system	specimen-rBGA-solution	NC
Tube test	4 µl rBGA + 50 µl specimen	4 µl PBS or NaCl + 50 µl specimen
Ortho BioVue	3,5 µl rBGA + 40 µl specimen	3,5 µl PBS or NaCl + 40 µl specimen
others	2 µl rBGA + 25 µl specimen	2 µl PBS or NaCl + 25 µl specimen

Prepare the specimen-rBGA-solution and the NC according to the table above, depending on the test system you intend to use. Vortex both mixtures briefly for 5 sec. Spin the liquid down in a table-top centrifuge for 5 sec at 8000 x g.

Incubate for 30 min at room temperature (19-25°C).

Indirect antiglobulin test

Analyse the specimen-rBGA-solution and the NC in a suitable test system (see manufacturer instructions for use).

4. Analysis and Troubleshooting

4.1. Analysis

Positive:

A specimen is positive for antibodies against the used rBGA if the reaction (agglutination) of the specimen with rBGA on antigen-positive red cells is negative in comparison with the NC.

Negative:

A specimen is negative for antibodies against the used rBGA if the strength of the reaction of the specimen with rBGA on antigen-positive red cells is not reduced in comparison with the NC.

Another antibody might be present in the specimen if the strength of the reaction is reduced but the agglutination is not completely inhibited. If the NC does not agglutinate, the result cannot be used for evaluation.

The guidelines for interpreting the strength of a reaction can be found in the instructions for use of the gel card manufacturer.

4.2. Troubleshooting

Problem	Possible Cause	Solution
Incomplete inhibition of the serum.	Antibodies with high titer Antibodies with high avidity	Increase the amount of protein step by step. Dilute the specimen (Note that additional antibodies may drop below detection level.)
	The specimen-rBGA-mix has not been sufficiently mixed (critical step).	Repeat the test and mix the specimens according to the instructions for use.

The functionality of rBGA has to be checked with a reference specimen. Please also consider the notes of the gel card manufacturer on troubleshooting and limitations. It is recommended to titrate all specimens to identify specimens with high titer.

5. Limitations and Characterization

5.1. Limitations

Incorrect execution of the instructions given in this instructions for use can lead to false results. In particular, wrong incubation times and temperatures as well as failure to mix rBGA and specimen may lead to false negative results.

Contamination of reagents or specimens, usage of reagents over their life-time, and usage of equipment or reagents not included or recommended may lead to false results.

In case of doubt other validated methods for the detection of anti-erythrocytic antibodies should be included in the evaluation.

5.2. Characterization

REF	Sensitivity [%]	n=	Specificity [%]	n=
R_Ch(a)	100	22	100	10
R_CR1_2	100	21	100	17
R_CROM	100	8	100	15
R_Do(a)	100	9	100	8
R_Do(b)	100	9	100	12
R_Fy(a)	100	13	100	9
R_Fy(b)	100	3	100	11
R_grKba	100	15	100	10
R_In(b)	100	3	100	7
R_JMH	100	33	100	78
R_klkba	100	5	100	6
R_Lu(a)_2	100	6	100	28
R_Lu(b)_2	100	6	100	21
R_LW(a)	100	3	100	5
R_Rg(a)	100	15	100	12
R_Sc1	100	5	100	51
R_Xg(a)	100	3	100	7
R_YCAD	100	4	100	22
R_Yt(a)	100	6	100	6
R_CD36	100	1	100	24
R_B07:02T	100	1	100	17

6. References

1. Seltsam A., Blasczyk R. (2009). Curr Opin Hematol. 2009 Nov;16(6):473-9. doi: 10.1097/MOH.0b013e3283319a06.

2. Seltsam A., et al. (2014). Transfusion. Jul;54(7):1823-30. doi: 10.1111/trf.12553.

7. Definition of Symbols and Abbrevations

rBGA	Recombinant blood group antigen
LOT	Batch code
REF	Catalogue number
⚠	Caution
	Consult instructions for use
	Use by date (YYYY-MM)
	Temperature limit
	Exclamation mark (GHS07) – Warning
	Manufacturer

imusyn GmbH & Co. KG · Feodor-Lynen-Str. 5 · 30625 Hannover, Germany · Tel: +49 511 563585-0 · Fax: +49 511 563585-229 · www.imusyn.de · info@imusyn.de

Σ	Contains sufficient for <n> tests
NaCl	0.9% sodium chloride solution
NK	Negative Control
PBS	Phosphate buffered saline pH 7.4

RUO	For resarch use only

Please check imusyn.de/IFU regularly for updates to these instructions for use.

Changes to the previous version are highlighted.

imusyn GmbH & Co. KG · Feodor-Lynen-Str. 5 · 30625 Hannover, Germany · Tel: +49 511 563585-0 · Fax: +49 511 563585-229 · www.imusyn.de · info@imusyn.de