Instructions for use

Sanquin Reagents B.V.
Plesmanlaan 125
1066 CX Amsterdam
The Netherlands

Phone: +31 20 5123599
Fax: +31 20 5123570
Reagents@sanquin.nl
www.sanquin.org/reagents

anti-Fy^a (IgG) monoclonal AGT method

REF K1236

IVD CE 0344

024_v04 07/2019 (en)

For professional use only

Blood grouping reagents for the detection of the Fy^a antigen on human red cells

General information
Sanquin monoclonal blood grouping reagents (clone numbers are mentioned on the corresponding certificate of analysis/release document and product label) are prepared from culture supernatants from stable hybridoma cell lines as first described by Köhler and Milstein (Nature 1975). This monoclonal reagent contains human IgG antibodies and has been specially selected and developed to provide a reliable alternative to polyclonal reagents. This reagent meets the requirements of the concerned standards and guidelines. Performance characteristics are mentioned in the release documents, which are supplied with the product upon request. The principle of the test is the agglutination technique, which is based on antigen/antibody reaction. The inclusion of positive and negative controls with each series of blood group determinations is strongly recommended.

Precautions
For in vitro diagnostic use only. Reagents should be stored at 2–8°C. Leaking or damaged vials must not be used. Reagents (unopened or opened) should not be used beyond the expiration date, which is printed on the label of the vial. NaN_3 0.1% (w/v) is used as preservative. The reagents cannot be assumed to be free from infectious agents. Care must be taken in the use and disposal of each container and its contents. Turbidity may indicate microbial contamination. To recognise reagent deterioration, testing of the reagent as part of the laboratory quality control program using appropriate controls is recommended. Waste-disposal, after completion of the test, should be performed according to your laboratory regulations.

Specimen collection and preparation
Blood samples should be withdrawn aseptically with or without the addition of anticoagulants. If testing of the blood samples is delayed, storage should be at 2–8°C.
Preparation of the specimen is described in the respective test procedures.

Test procedure
Indirect Antiglobulin Test (AGT method)
Tube requirements: round bottom glass tubes; size 75 x 10/12 mm.

1. Prepare a 3–5% cell suspension of erythrocytes to be tested, suspended in isotonic saline.
2. Add to a test tube:
   - 1 drop of AGT reagent
   - 1 drop of the 3–5% cell suspension

   and mix well.
3. Incubate in a water bath for 15 minutes at 37°C.
4. Wash the tube 3 times in an excess of isotonic saline. Decant the last wash completely.
5. Add 2 drops of polyspecific anti-human globulin serum and mix well.
6. Centrifuge for 20 seconds at 1000 rcf or for a time appropriate to the calibration of the centrifuge.
7. Resuspend the cells by gentle agitation and read macroscopically for agglutination.
8. If there is no visible agglutination, add 1 drop of Coombs Control Cells and repeat steps 6 and 7; the reaction should now be positive. If the test remains negative the result is invalid and the test should be repeated.

Interpretation
A positive reaction (i.e. agglutination) indicates the presence of the corresponding antigen. A negative reaction (i.e. no visible agglutination) indicates the absence of the corresponding antigen.

Occurrence	Caucasians	Negroids
Fy^a	66%	10%

Limitations
Unexpected positive results due to: pseudoagglutination, autoagglutination, mixed field reaction.
Unexpected negative or weak results due to: weak antigens, mixed field reaction, decreased activity of the reagent.
Antigen variant cells may produce unexpected positive or negative reactions with samples previously typed with blood grouping reagents of polyclonal or other cell line-derived monoclonal sources.
False positive or false negative results may occur through contamination of test materials or any deviation from the recommended technique.
Red cells coated in vivo with IgG antibodies and/or complement components will be agglutinated by the polyspecific anti-human globulin serum and as such provoke a false positive determination of blood group antigens. To eliminate this possibility, a Direct Antiglobulin Test (DAT) should be performed on the sample prior to typing of blood group antigens.
Ineffective washing of the red cells being tested can result in false negative results due to the neutralisation of the polyspecific anti-human serum by proteins (IgG) still present in the tube.

Sanquin blood grouping reagents have been optimized for use by the technique recommended in this package insert. Unless otherwise stated their suitability for use by other techniques must be determined by the user.

References

1. Race R.R. and Sanger R.; Blood Groups in Man, 6th ed. Oxford Blackwell Scientific Publishers 1975.
2. Issitt P.D.; Applied Blood Group Serology, 3rd ed. Montgomery Scientific Publications, Miami, Florida, USA, 1985.
3. Daniels G.; Human Blood Groups. Blackwell Science Ltd. 1995.
4. Reid M.E. and Lomas-Francis C.; The Blood Group Antigen Facts Book. Facts Book Series, 1997.
5. Mollison P.L. et al.; Blood Transfusion In Clinical Medicine, 9th ed. Blackwell, Oxford, 1993.

Sanquin products are guaranteed to perform as described in the original manufacturer's instructions for use.
Strict adherence to the procedures, test layouts and recommended reagents and equipment is essential. Sanquin declines all responsibility arising from any deviation thereof.