REAGENT RED BLOOD CELLS
PANOCELL® –10, FICIN-TREATED
For Identification of Unexpected Antibodies

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US LICENSE 886
MADE IN USA

IMMUCOR

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Intended Use:
Ficin-Treated Panocell Reagent Red Blood Cells are intended for use in the identification of unexpected red blood cell blood group antibodies.

Summary of the Test:
Ficin modifies the erythrocyte membrane by destroying or altering select membrane components or component environments. As a consequence, interactions between some antibodies and their respective antigens are strengthened while others are abolished. Solutions of proteolytic enzymes, such as ficin, have been used since the late 1940s in serological testing. The reactions of Rhesus, Lewis, Kidd and P blood group antibodies are usually enhanced in techniques employing enzyme-treated red blood cells. [1-5] However, some antibodies, most notably the commonly encountered specificities of the MN and Duffy systems, fail to react with ficin-treated red blood cells altogether. [1-8]
Panocell-10, Ficin-Treated, is manufactured as enzyme-premodified and correlates to Panocell-10, untreated, reagents of the same lot number. Ficin-treated red blood cells are tested in parallel with, or subsequent to, unmodified red blood cells.

Principle of the Test:
Serum is systematically tested against Panocell reagent red blood cells (untreated or ficin-treated). Agglutination of one or more of the reagent red blood cells at any phase, or hemolysis at the saline or incubated phases of testing, constitutes a positive test and is the result of a reaction between an antigen and its respective antibody. No agglutination or no hemolysis may indicate either the absence of antibody (providing the test red blood cells possess the corresponding antigen) or that an antibody, if present, is in concentrations too low to be detected by the serologic technique employed. In the case of ficin-treated red blood cells, a negative reaction also may indicate that the antibody present is directed against an enzyme-sensitive antigen.

Reagents:
Panocell-10, Ficin-Treated: a 12-vial set of 10 vials of red blood cells, plus one vial each of Ficin Solution and Ficin Control.
Each cell vial contains a 2-4% suspension of single donor, group O red blood cells that have been treated with a ficin solution. Following exposure to enzymes, the red blood cells are washed and suspended in a buffered preservative solution containing adenosine and adenine to retard hemolysis and/or loss of antigenicity during the dating period.
Chloramphenicol (0.25 mg/mL), neomycin sulfate (0.1 mg/mL) and gentamycin sulfate (0.05 mg/mL) have been added as preservatives. The diluent does not interfere with complement mediated hemolysis.
Ficin-treated reagent red blood cells are supplied in reagent sets as companions to Panocell reagents of matched lot numbers. (Refer to direction circulars accompanying untreated reagents IC0316 for discussion of their use.) The red blood cells used are the same as those used in the untreated reagents. The Master List accompanying each lot indicates the donor code and antigenic composition of each single donor red blood cell reagent prior to ficin treatment.
Ficin Solution, supplied with Panocell-10, Ficin-Treated, should be used to treat patient or donor red blood cells for inclusion as an autologous control in identification tests employing ficin-premodified reagents. **Note:** Precipitate is sometimes found in the bottom of Ficin Solution vials and is not an indication of product deterioration. Quality control testing is necessary to detect product deterioration. Ficin Control (lectin of *Glycine max* var. *soja*) is used to verify that autologous red blood cells have been sufficiently treated with enzymes prior to testing. Both reagents contain sodium azide (0.1% (w/v) final concentration) as a preservative.

Storage:
- Store at 1° to 10°C when not in use.
- Do not freeze or expose to elevated temperatures.
- Do not use beyond expiration date which is expressed as CCYY-MM-DD (year-month-day).

Precautions and Warnings:
- For *in-vitro* diagnostic use.
- For laboratory professional use only.
- Avoid contaminating this product during use.
- Do not use contaminated reagents.
- Do not use leaking vials.
- Contamination will adversely affect the product's performance during its shelf-life.
- Do not use unlabeled vials.
- No U.S. standard of potency.
- Reagent red blood cells should not be used if the cells darken, spontaneously clump or if there is significant hemolysis.
- Slight hemolysis may occur with age. In this instance, the red blood cells may be washed and suspended in saline immediately prior to use.
- Suspend the red blood cells before use by gently inverting each vial several times.

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Ficin Solution and Ficin Control contain 0.1% (w/v) sodium azide.
Warning: Sodium azide may react with lead and copper plumbing to form explosive compounds. If discarded into the sink, flush with a large volume of water to prevent azide build-up.

CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIAL FROM WHICH THIS PROUDCT WAS DERIVED WAS FOUND NEGATIVE WHEN TESTED IN ACCORDANCE WITH CURRENT FDA REQUIRED TESTS. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS.

Handle and dispose of reagent as if potentially infectious.

LATEX

The packaging of this product (dropper bulbs) contains dry natural rubber.

Specimen Collection and Preparation:
Serum or plasma may be used in antibody identification procedures. Plasma anticoagulants may interfere with the detection of complement-binding antibodies. Fibrin clots may also develop and interfere in tests employing plasma.

Draw a blood specimen using an acceptable phlebotomy technique. Testing should be performed as soon as possible to minimize the chance that falsely positive or falsely negative reactions will occur due to improper storage or contamination of the specimen. Should delays in testing occur, specimens should be stored at 1-10°C. Alternatively, serum or plasma can be separated from red cells and stored frozen. Weakly reactive antibodies may deteriorate and become undetectable in samples stored at room temperature for several days before testing or in samples stored for prolonged periods at 1-10°C. Do not use samples drawn into tubes with neutral gel separators. False-positive results may occur with such samples.

Procedure:
Materials Provided:
1. Panocell-10, Ficin-Treated REF 0002385 containing matched lots of untreated and ficin-treated red blood cells from the same donor, supplied in dropper vials, ready for use
2. Panocell® Master List

Additional Materials Required for Procedures Using Ficin-Treated Red Cells But Not Provided:
1. Donor or patient sample
2. 10 x 75 mm or 12 x 75 mm test tubes
3. Test tube rack
4. Transfer pipettes
5. Isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline, pH 6.5-7.5
6. Anti-Human Globulin (polyspecific or ati-IgG)
7. Antiglobulin control cells (cells sensitized with IgG) (e.g.,Immucor Checkcell)
8. 37°C water bath or dry heat incubator
9. Serologic centrifuge*
10. Interval timer*
11. Marking pen
12. Optical aid such as a hand lens, a concave mirror or a microscope (optional)

*It is the user's responsibility to validate an accessory device (either listed or otherwise) for its intended use. Validation results should be maintained as part of the laboratory's records for review by regulatory agencies.

Test Method: The procedure detailed below is intended as a guideline for antibody identification procedures using enzyme-premodified red blood cells. (Consult the package inserts accompanying untreated Panocell IC0316 for the performance of tests using these reagents.) It may be desirable to modify this procedure to comply with the requirements or in-house standard operating procedures of the particular laboratory. It is not recommended that potentiating agents be used with enzyme-premodified red blood cells.
1. Label 1 test tube for each Panocell reagent to be tested, and if performed, one additional tube for an autologous control.*

Key:
Underline = Addition or significant change; ▲ = Deletion of text

2. Prepare ficin-treated autologous control cells according to the protocol outlined below. (See **Autologous Control Preparation**.)
3. Place 2-3 drops of the test serum or plasma into each of the labeled tubes. Adding 3 drops may enhance reactivity.
4. Gently invert each reagent red blood cell vial several times to achieve a complete resuspension of the red blood cells.
5. Add 1 drop of each of the reagent red blood cells to the appropriately labeled tubes. If an autologous control is to be tested in parallel, add 1 drop of a 2-4% suspension of the ficin-treated red blood cells (prepared in step 2) to the appropriate tube.
6. Mix the contents of each tube thoroughly, then incubate the tubes at 36-38°C for 15 to 30 minutes. **NOTE:** If desired, all tubes may be centrifuged and examined for agglutination or hemolysis prior to the 37°C incubation.
7. Centrifuge each tube.** Examine the supernatant fluids for hemolysis. Gently suspend each red blood cell button and examine for agglutination. Record results.
8. Wash the red blood cells a minimum of three times with isotonic saline, being careful to decant completely after each wash.
9. Add Anti-Human Globulin to each tube in the amount specified by the manufacturer's product insert.
10. Mix the contents of each tube thoroughly.
11. Centrifuge each tube.** Gently suspend each red cell button and examine macroscopically for agglutination. Record results.
12. Confirm the validity of all negative reactions with IgG-sensitized antiglobulin control red blood cells.

Autologous Control Preparation:

1. Dilute 0.1 mL of Ficin Solution in 0.9 mL of saline.
2. Add 1 drop of a 2-5% suspension of the autologous red blood cells to a tube marked TEST. Add another drop to a tube marked CONTROL.
3. Add one drop of the diluted Ficin Solution to each tube and mix the contents of each tube thoroughly.
4. Incubate the tubes at 36-38°C for 10-15 minutes.
5. Wash the red blood cells at least three times with isotonic saline.
6. Add 2 drops of Ficin Control to the tube marked CONTROL. Mix the contents.
7. Centrifuge the CONTROL tube.**
8. Gently suspend the red blood cell button and examine for agglutination. Red blood cells that have been adequately treated with Ficin should produce 3-4+ results with Ficin Control. If the desired reactions are obtained, the red blood cells in the TEST tube are ready for use in identification procedures. If weak reactions are obtained with in the CONTROL test, repeat the premodification procedure.

*It is important to know if a serum reacts with autologous red blood cells to determine whether alloantibodies or autoantibodies are present. This is particularly important when enzyme-premodified red blood cells are used for antibody identification since enzyme-treated red blood cells may react more readily with autoantibodies (particularly autoanti-I) than with untreated red blood cells.

** Suggested centrifugation time: 15-30 seconds at 900-1000 x g or a time, appropriate for the centrifuge used, that produces the strongest reaction of antibody with antigen-positive red blood cells, yet allows easy suspension of antigen-negative red blood cells.

Stability of Final Reaction:

Following centrifugation, all tests should be read immediately, and results interpreted without delay. Delays may result in dissociation of antigen-antibody complexes leading to falsely negative, or at most, weakly positive reactions.

Quality Control:

In addition to visual inspection for evidence of deterioration, the reactivity of the red blood cells may be checked periodically by testing antigens likely to deteriorate, such as Le^a, with weakly reactive antibodies of the same specificity. If such antigens are found nonreactive, the product should not be used.

Interpretation of Results:

The principles of antibody identification using ficin-treated red blood cells are similar to those for tests using untreated red blood cells. Consult the package insert for Panocell-10, untreated, reagents IC0316 included in the Ficin-Treated Reagent Set for a discussion of the basic principles of identification.

Positive test using ficin-treated red blood cells: Agglutination of any, or all, red blood cell reagent(s) at the antiglobulin phase, or agglutination or hemolysis at 37°C.

Negative test using ficin-treated red blood cells: No agglutination or hemolysis of any cell reagent.

Reactions obtained with ficin-treated red blood cells may, in many instances, differ from those obtained with untreated red blood cells. The reactions of certain antibodies are enhanced, while others are diminished.

Antibodies whose reactions are enhanced by enzymes	Anti-D, -C, -c, -E, -e, -f, $-Jk^a$ $-Jk^b$, $-Le^a$, $-Le^b$, -I, -IH, $-Tj^a$
Antibodies whose reactions are diminished or abolished	Anti -M, -N, -S, -s, $-Fy^a$, $-Fy^b$, $-Xg^a$, -Pr, -Ch, -Rg, -JMH

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Limitations:

Falsely positive or falsely negative test results can occur from bacterial or chemical contamination of test materials, inadequate incubation time or temperature, improper centrifugation, inadequate washing of red cells, improper storage of test materials and omission of antiglobulin serum or test serum. Falsely negative results may be obtained if an inappropriate serum-to-cell ratio is used. [9] It is important to perform antibody identification procedures using an optimum serum-to-cell ratio. The amount (number of drops) of serum employed will depend on the percent suspension of red blood cells used or the delivery volume of the dropper.

Infrequently, positive results may occur in the presence of antibodies directed to components of the red blood cell diluent. These unwanted reactions can usually be avoided by washing the reagent red blood cells in saline prior to testing.

Warm- or cold-reactive autoantibodies are enhanced in tests employing enzyme-premodified red blood cells.

Prolonged exposure of patient or donor red blood cells to the Ficin Solution supplied with Panocell-10, Ficin-Treated, will lead to overtreatment of the red blood cells. Red blood cells that have been overtreated may spontaneously aggregate in serum or saline making test interpretation difficult.

Ficin-treated red blood cells may be more prone to lysis by hemolytic antibodies (certain examples of anti-Vel, anti-Tj^a, anti-Jk^a, anti-Le^a) than untreated red blood cells of the same donor.

Enzyme-premodified red blood cells should not be the sole reagents used for antibody detection.

The reactivity of Reagent Red Cells may diminish over the dating period. The rate at which antigen reactivity (i.e., agglutinability) is lost is partially dependent upon the individual donor characteristics that are neither controlled nor predicted by the manufacturer.

No one test method is capable of detecting all unexpected red blood cell antibodies.

The red blood cells used to prepare this reagent will carry antigens that may not be defined by the manufacturer, therefore, it is possible to obtain positive reactions with this reagent that do not match the profiles of any reagents shown on the Master List.

The user is responsible for validating the use of Panocell-10, Ficin-Treated in methods other than the manual tube method described in this insert.

Specific Performance Characteristics:

Prior to release, each lot of Immucor Reagent Red Blood Cells, unless otherwise indicated, is tested by two independent laboratories using no less than two donor sources of antibody (except where precluded by the rarity of the antibodies) to confirm the presence or absence of all blood group antigens specified on the Master List. The performance of this product is dependent upon adhering to the insert's recommended methodology. All red blood cell suspensions are tested and shown to have a negative direct antiglobulin test using polyspecific Anti-Human Globulin. Ficin-treated reagent red blood cell sets meet the requirements of the FDA for reagent red cells for use in the identification of unexpected antibodies. The expiration date for these products is set at 67 days from the date of manufacture which is the earliest date that blood is withdrawn from any donor used in a component of the product.

For additional information or for technical support, contact Immucor at 855-IMMUCOR (466-8267) (US/Canada).

To get a Certificate of Analysis (CoA), an electronic copy of the IFU or, a Safety Data Sheet please go to www.immucor.com and enter Customer Login or contact your local Customer Service:

Bibliography:

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Symbols Glossary:

The Symbols Glossary (ID No. 400) is provided electronically at http://adextranet.immucor.com/EN/Pages/PackageInsers.aspx.