



**Com-DF, ANTI-Rh D:R-PE/ANTI-HbF:FITC CONJUGATED
DUAL STAINING REAGENT, READY TO USE
CERTIFICATE OF ANALYSIS**

CATALOG NUMBER:	MAB3435F-100T	QUANTITY:	100 T
LOT NUMBER:		CONCENTRATION:	0.02 mg/mL
HOST/ISOTYPE:	Ms IgG1		

BACKGROUND: Currently, there is a world shortage of Rh D immunoglobulin for immunoprophylaxis. Administration of the appropriate dose is encouraged ^(NHMRC Guidelines, 1996) to ensure adequate protection against alloimmunization while conserving a valuable resource. To determine the appropriate dose, it is necessary to quantitate the magnitude of a suspected FMH and administer Rh D immunoglobulin according to the extent of the fetal bleed. The required dose is estimated on the basis that 20µg of Rh D immunoglobulin will suppress immunization by 1mL D-positive red cells (2mL fetal blood). Pack sizes for Rh D immunoglobulin vary. In Australia, 125µg is considered to be sufficient to suppress immunization by up to 12mL of fetal blood. ^(NHMRC Guidelines, 1996). Assuming a maternal blood volume of 5000ml, a hemorrhage of this magnitude would be detectable in the maternal circulation as approximately 0.25% of the red cell population or 1/400 cells.

The use of flow cytometry to quantitate minor populations of Rh D and HbF positive cells has been explored by a number of workers ^(Nance et al, 1989-Lloyd-Evans, et al 1996). The capacity of the flow cytometer to analyze a large number of cells in a short period of time enables accurate quantitation of FMH at clinically relevant levels. **Com-DF** is designed for use with a flow cytometer. The test is simple to perform, rapid, reliable and objective.

SPECIFICITY: This reagent recognizes both the Rh D antigen found on the red blood cells (RBC) of Rh D positive individuals and Fetal Hemoglobin (HbF) of human red blood cells.

This test is confined to use on maternal blood samples from Rh D negative women while pregnant or following delivery of Rh D positive babies. FMH during pregnancy may be caused by threatened miscarriage, chorionic villus sampling, placenta abruption, abdominal trauma or trauma induced by external cephalic version. FMH also may occur as a result of ectopic pregnancy, spontaneous abortion or termination of pregnancy. The test should be performed on maternal blood after any such event ^(NHMRC Guidelines, 1996). In addition, the magnitude of FMH should be estimated following delivery of Rh D positive infants ^(NHMRC Guidelines, 1996). The test should be carried out soon after the precipitating event or delivery in order to permit the timely administration of the appropriate amount of Rh D immunoglobulin to prevent alloimmunization occurring in the mother.

This reagent is designed for the detection and quantitation of Rh D positive and HbF positive RBC arising in the circulation of mothers as a consequence of fetomaternal hemorrhage (FMH). The procedure requires prefixation and permeabilization of the cells followed by a single staining step and analysis using a flow cytometer.

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- APPLICATIONS:** Flow cytometry, see enclosed protocol.
- SPECIES REACTIVITY:** Human, other species not yet tested.
- PRESENTATION:** Purified mouse immunoglobulin in phosphate buffered saline, pH 7.4, 0.1% sodium azide, and 0.2% bovine serum albumin.
- STORAGE/HANDLING:** Store at 2-8°C, valid for 18 months. **DO NOT FREEZE.** Protect from light.
- REFERENCES:**
- Guidelines for the use of RhD immunoglobulin (anti-D) in obstetrics. 1996. Prepared by the National Health Advisory Committee Working Party on guidelines for the use of RhD immunoglobulin (anti-D). Available from the Publications Officer, NHMRC, GPO Box 9848, Canberra, ACT 2601.
- Nance SJ, Nelson JM, Arndt PA, Lam H-TC and Garratty G. 1989. Quantitation of fetal-maternal hemorrhage by flow cytometry. *Am. J. Clin. Pathol.* 91:288- 292.
- Bayliss KM, Kueck BD, Johnson ST, Fueger JT, McFadden PW, Mikulski D and Gottschall JL. 1991. Detecting fetomaternal hemorrhage: a comparison of five methods. *Transfusion* 31: 303-307.
- Nelson M, Popp H, Horky K, Forsyth C and Gibson J. 1994. Development of a flow cytometric test for the detection of D-positive cells after fetomaternal hemorrhage and survey of the prevalence in D-negative women. *Immunohaematology* 10:55-59.
- Lloyd-Evans P, Kumpel BM, Bromelow I, Austin E and Taylor E. 1996. Use of a directly conjugated monoclonal anti-D (BRAD-3) for quantification of fetomaternal hemorrhage by flow cytometry. *Transfusion* 36: 432-437.

PROTOCOL:

1. Collect maternal blood into EDTA anticoagulant. (Sample must be drawn prior to administration of Rh D immunoglobulin.)
2. Mix thoroughly and dilute the blood 1/10 in wash buffer (see Reagent Preparation), vortex gently and dispense 20 µL of the diluted blood into the bottom of a flow cytometer tube.
3. Add 1 mL of cold freshly prepared Prefixation solution (see Reagent Preparation). Vortex and incubate for 10 minutes at room temperature.
4. Add 2mL of wash buffer, mix and centrifuge to achieve gentle sedimentation of RBC. Repeat for a total of 3 washes.
5. Carefully decant or aspirate by vacuum suction discarding the supernatant.
6. Resuspend the RBC pellet in 0.5 mL of Permeabilization solution (see Reagent Preparation) by gentle vortexing. Incubate for 3-5 minutes at room temperature.
7. Wash once with 2 mL of wash buffer.

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8. Carefully discard or aspirate the supernatant.
9. Add 50µL of **Com-DF™** to the RBC pellet.
10. Vortex gently and incubate at room temperature in the dark for 15 minutes.
11. Add 2 mL of wash buffer, mix and centrifuge at 600 x g.
12. Decant or aspirate, discarding the supernatant.
13. Resuspend the RBC pellet in 0.6mL of fixing solution (see Reagent Preparation) and proceed to data acquisition by flow cytometry. Store cells at 4°C in the dark prior to analysis if preferred.

ANALYSIS:

Standards: To optimize your flow cytometer settings, use of one of the following standards is recommended.

Chemicon Fetal Control Kit (FT100), which consists of 3 stabilized, prepared controls of human RhD negative adult erythrocytes, supplemented with human RhD positive cord blood erythrocytes. The three controls represent no fetal cells, low and high levels of fetal RhD positive RBC.

Blood samples from ABO compatible, D-negative and D-positive donors arranged to give:

- 100% D-negative cells
- a mixture comprising 99.75% D-negative and 0.25% D-positive cells

These standards should be prepared fresh and 20 µL of a 1:10 dilution in wash buffer run in parallel with each batch of samples tested.

Data acquisition: For each test, collect 50,000 events gating on the singlet RBC population.

Data analysis: The vertical scale of fluorescence intensity histograms should be adjusted so that small HbF and D-positive populations can be visualized. Results should be expressed to 2 decimal places.

LIMITATIONS OF THE TEST:

1. This test has the capacity to quantify FMH.
2. In cases of ABO incompatibility between mother and child, the natural ABO antibodies of the mother may destroy fetal cells in the maternal circulation before testing is performed

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC
PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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