Product Insert for FITC conjugated Anti-D Reagent for Determination of Feto-Maternal Haemorrhage

CE

IVD

NHS Blood and Transplant

National Blood Service
BRAD 3 anti-D FITC conjugated reagent
Product Code 9433FI
for in vitro use only
Lot: FBr3.28

Material provided:

- 0.51mL +/- 0.05ml BRAD 3 anti -D FITC conjugated reagent in an amber bottle.
- Contains 0.1 mg/ml antibody in PBS pH 7.2-7.4, 0.099% sodium azide and 1% bovine serum albumin (BSA).

Specifications:

- This reagent has been formulated to contain anti-D suitable for flow cytometry during the lifetime of the reagent if stored as recommended.
- BRAD 3 is a human IgG3 anti-RhD (ISBT No. 4001) monoclonal antibody produced by an EBV transformed B cell line derived from the peripheral blood of an immunised RhD negative donor. This monoclonal anti-D reacts as an indirect agglutinin with all RhD positive red cells tested except those of the rare D^{VI} or R_o^{har} types.
- RhD positive infants born to RhD negative women may suffer from haemolytic disease of the newborn. The disease can be prevented by administration of anti-D post partum or antenatally. The dosage of anti-D required depends on the size of feto-maternal haemorrhage. By flow cytometry, FITC-BRAD 3 can be used to quantitate accurately the number of RhD positive cells in a mixture of RhD positive and negative cells, and thereby estimate the size of feto-maternal haemorrhage by analysis of a maternal blood sample.
- FITC conjugated BRAD 3 has also been used in conjunction with R-phycoerythrin conjugated BRIC 256 (anti-Glycophorin A) in a dual labelling flow cytometry method for FMH quantitation.
- Each lot of this reagent has been assessed using NBS - Reagents Quality Assurance procedures.

Recommended Use:

- BRAD 3 FITC is supplied ready for use at 5 µl (or can be prepared as a 1 in 10 dilution in PBS and 50µl used) per test. To a pellet of 10^7 washed packed erythrocytes add 50 µl of PBS pH 7.2-7.4. Add 5 µl of BRAD 3 FITC. Mix. Incubate at 37°C in the dark for 30 min. Wash the cells once in PBS. Analyse by flow cytometry.
- BCSH Guidelines for FMH are published in Chapman JF (1999) Working party of the BCSH Blood Transfusion and General Haematology Task Forces (1999) Transfusion Medicine **9**, 87-92.
- The variability of fluorescence of rr cells (RhD negative) when labelled with FITC anti-D (such as BRAD 3 FITC) is such that for accurate interpretation of results it is necessary to use as a negative control an antibody unreactive with human red cells irrespective of their RhD type, such as AEVZ 5.3 FITC.

Limitations:

- This reagent is not qualified for use as an anti-D grouping reagent.
- Suitability for use in other techniques must be determined by the user.

Storage and stability:

- Store at 2-8°C.
- Protect from the light.

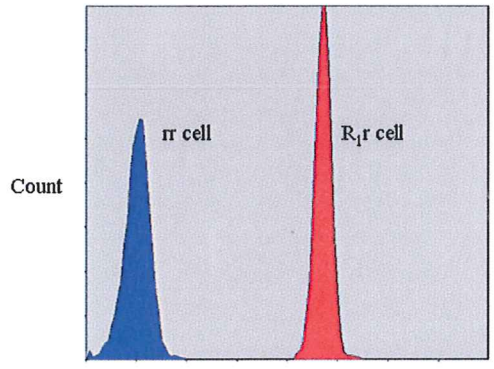
Precautions:

- Do not freeze.
- Protect from contamination.
- Keep stored in the amber bottle.
- The reagent should not be used if turbid or if a precipitate, gel or particles are present.
- The reagent has been prepared using a diluent containing 1% BSA and 0.099% sodium azide.

Quality Control Data on this Lot
Unlabelled erythrocytes showed a mean peak channel fluorescence of: 0.295
Lot no. FAEVZ 5.3-11 of AEVZ5.3 FITC used as control for measurements below:
Peak channel fluorescence with rr and R_1r red cells

Red cell	BRAD3 FITC (Lot FBr3.28) (Product code 9433FI)	AEVZ5.3 FITC (Lot FAEVZ 5.3-11) (Product code 9442FI)
rr	0.33	0.37
R_1r	22.6	0.36

BRAD 3 FITC Lot FBr3.28 with R_1r and rr cells



Interpretation of Results

- The analysis of the results should be according to the Guidelines for the Estimation of Fetomaternal Haemorrhage, which are published by the Working Party of the British Committee for Standards in Haematology, Transfusion Taskforce. BCSH FMH guidelines 2009.
- For NBS centres please refer to the Management Process Description MPD444
- Lloyd-Evans *et al*, (1995), Use of a FITC-conjugated monoclonal anti-D (BRAD-3) for quantitation of fetal leaks by flow cytometry, Transfusion Medicine **5**, suppl. 1, 23
- Lloyd-Evans *et al*, (1996), Use of a directly conjugated monoclonal anti-D (BRAD-3) for quantification of fetomaternal haemorrhage by flow cytometry, Transfusion, **36**, 432-437.
- Lloyd-Evans *et al*, (1999), Detection of weak D and D^{VI} red cells in D-negative mixtures by flow cytometry: implications for feto-maternal haemorrhage quantification and D typing policies for newborns, British J. Haematol. **104** 621-625.

National Blood Service Reagents are produced at the following Centres

NBS Liverpool
14 Estuary Banks,
Speke
Liverpool L24 8RB
Phone 0151 268 7157

IBGRL
NHSBT North Bristol Park,
Filton
Bristol BS34 7QG
Phone 0117 921 7500

Product Insert for FITC Conjugated Negative Control Reagent for Determination of Feto-Maternal Haemorrhage

CE

IVD

National Blood Service

NHS Blood and Transplant

AEVZ 5.3 FITC conjugated negative control reagent
Product Code 9442FI

for in vitro use only

Lot: FAEVZ5.3-13

Material provided:

- 0.51mL +/- 0.05ml AEVZ 5.3 FITC conjugate in an amber bottle.
- Contains 0.1 mg/ml antibody in PBS pH 7.2-7.4, 0.099% sodium azide and 1% bovine serum albumin (BSA).

Specifications :

- This reagent has been formulated to be suitable for flow cytometry during the lifetime of the reagent if stored as recommended.
- AEVZ 5.3 is a human IgG3 engineered monoclonal antibody.
- RhD positive infants born to RhD negative women may suffer from haemolytic disease of the new-born. The disease can be prevented by administration of anti-D post partum or antenatally. The dosage of anti-D required depends on the size of feto-maternal haemorrhage. By flow cytometry, FITC-BRAD 3 can be used to quantitate accurately the number of RhD positive cells in a mixture of RhD positive and negative cells, and thereby estimate the size of feto-maternal haemorrhage by analysis of a maternal blood sample. The variability of fluorescence of rr cells (RhD negative) when labelled with FITC anti-D is such that for accurate interpretation of results it is necessary to use as a negative control an antibody unreactive with human red cells irrespective of their RhD type, such as AEVZ 5.3 FITC.
- Each lot of this reagent has been assessed using NBS - Reagents Quality Assurance procedures.

Recommended Use:

- AEVZ5.3 FITC is supplied ready for use at 5 µl per test (or can be prepared as a 1 in 10 dilution in PBS and 50µl used) as a negative control alongside BRAD-3 FITC. To a pellet of 10^7 washed packed erythrocytes add 50 µl of PBS pH 7.2-7.4. Add 5 µl of AEVZ5.3 FITC. Mix. Incubate at 37°C in the dark for 30 min. Wash the cells once in PBS. Analyse by flow cytometry.
- BCSH Guidelines for FMH are published in Chapman JF (1999) Working party of the BCSH Blood Transfusion and General Haematology Task Forces (1999) Transfusion Medicine **9**, 87-92.

Limitations:

- Suitability for use in other techniques must be determined by the user.

Storage and stability:

- Store at 2-8°C.
- Protect from the light.

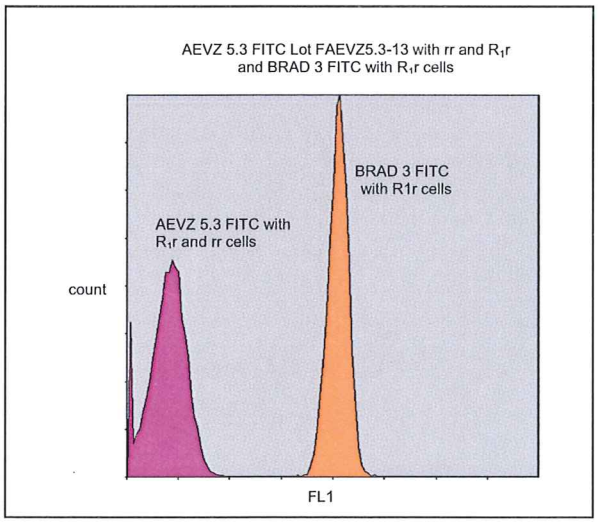
Precautions:

- Do not freeze.
- Protect from contamination.
- Keep stored in the amber bottle.
- The reagent should not be used if turbid or if a precipitate, gel or particles are present.
- The reagent has been prepared using a diluent containing 1% BSA and 0.099% sodium azide.

Quality Control Data on this Lot

Unlabelled erythrocytes showed a mean peak channel fluorescence of: 0.302 with rr cells and 0.285 with R1r cells. Lot no. FBr3. 28 of BRAD3 FITC was used as a control for measurements below:
Peak channel fluorescence with rr and R_1r red cells

Red cell	BRAD3 FITC 3 (Lot FBr3.28) (Product code 9433FI)	AEVZ 5.3 (Lot FAEVZ5.3-13) (Product code 9442FI)
rr	0.345	0.356
R_1r	17.6	0.351



Interpretation of Results

- The analysis of the results should be according to BCSH Guidelines for FMH which are published in Chapman JF, Working party of the BCSH Blood Transfusion and General Haematology Task Forces (1999) Transfusion Medicine **9**, 87-92.
- For NBS centres please refer to the Management Process Description MPD/DDR/RC/034
- Lloyd-Evans P, Austin EB, Gilmour JEM, Scott ML (1999) Use of a negative control antibody in the quantitation of feto-maternal haemorrhage by flow cytometry. Transfusion Medicine 9 suppl. 1:33. 9.The analysis of the results should be according to BCSH Guidelines for FMH which are published in Chapman JF, Working party of the BCSH Blood Transfusion and General Haematology Task Forces (1999) Transfusion Medicine **9**, 87-92.
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