

**International Blood Group
Reference Laboratory**

500 North Bristol Park
Northway
Filton
Bristol
BS34 7QH

Antigen	Human Blood Group Rh D (ISBT No. 4001) CD 240D
Clone	BRAD 3
Product Code	9433
Immunoglobulin Class	Human IgG3, kappa light chain

**Protein Development
and Production Unit**

Tel: +44 (0)117 921 7500

Fax: +44 (0)117 912 5796

Website: <http://ibgri.blood.co.uk>

Email: enquiries.IBGRL@nhsbt.nhs.uk

Antigen Description and Distribution

The Rh D antigen (Rh_H or Rh_o) is clinically the most important of the Rh blood group system. It is expressed on the extracellular loops of a transmembrane polypeptide of around Mr 30000¹. Estimated numbers of Rh D sites recognised by BRAD-3 on Rh D positive cells are between 10000 to 14000 on CDe/cde (R_{1r}) cells and 27000 to 33000 on cDE/cDE (R_{2R2}) cells². Rh D positive infants born to Rh D negative women may suffer from haemolytic disease of the newborn. The disease can be prevented by administration of anti-D post partum or antenatally. Dosage of anti-D depends on the size of feto-maternal haemorrhage (FMH). In humans the Rh D antigen is expressed solely on the erythrocytes of Rh D positive individuals. 85% of Caucasians are Rh D positive.

Clone

BRAD 3 is produced by an EBV transformed B cell line derived from the peripheral blood of an immunised Rh D negative donor³. This monoclonal anti-D reacts as an indirect agglutinin with all Rh D positive red cells tested except those of the rare D^{VI} or R_o^{har} types (pattern 13^{4,5}). BRAD 3 was submitted to the third international workshop on monoclonal antibodies against human red cells, Nantes 1996⁶. By flow cytometry, FITC-BRAD 3 can be used to quantitate accurately the numbers of Rh D positive cells in a mixture of Rh D positive and negative cells, and thereby estimate the size of feto-maternal haemorrhage (FMH)^{7,8}. BRAD 3 can also discriminate weak D in feto-maternal bleeds where the site/cell numbers are above 1000 RhD sites^{9,10}. 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^{11,12}. BCSH Guidelines for FMH have been published^{13,14}. When measuring the variance of rr cells in terms of background binding of FITC conjugated IgG, the use of a negative control FITC-labelled antibody should be used in parallel with FITC anti-D on clinical samples¹⁵. For estimation of FMH, the introduction of the use of BIRMA 17C conjugated to R-Phycoerythrin (PE) in 2014 showed that the removal of granulocytes during flow cytometry which may otherwise interfere in the assay and thus affect the final calculated bleed, gives a more accurate result. PE conjugated BIRMA 17C is used in conjunction with FITC conjugated BRAD 3 as a two (dual) colour reagent used for FMH quantitation as well as AEVZ 5.3 FITC negative control^{16,17}.

References

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