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

Antigen Description and Distribution

The Rh D antigen (Rh_1 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 30000[1]. 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_2R_2) cells[2]. 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 donor[3]. 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 1996[6]. 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 samples[15]. 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]. BRAD 3 has been used by flow cytometry to analyse D-positive erythrocyte sub-populations[18].

References

1. Cartron, J-P, (1994) Blood Reviews **8**, 199-212.
2. Jones J. *et al*, (1996) Vox Sanguinis **71**, 176-183.
3. Kumpel B. *et al*, (1989) Br J Haematol **71**, 125-129.
4. Jones J. *et al*, (1995) Transfusion Medicine **5**, 171-184.
5. Jones J. *et al*, (1996) Vox Sanguinis **70**, 173-179.
6. Rouger P Muller JY (eds) (1997) Proceedings of the third International workshop and symposium on monoclonal antibodies against human red cells and related antigens, Nantes 1996.
7. Lloyd-Evans P. *et al*, (1995) Transfusion Medicine **5**, suppl 1, 23.
8. Lloyd-Evans P. *et al*, (1996) Transfusion, **36**, 432-437.
9. Lloyd-Evans P. *et al*, (1999) Transfusion Medicine **9,** 155-160.
10. Kumpel B. (2001) Transfusion **41**, 1059-1063.
11. Lloyd-Evans P. *et al*, (1999) Br J Haematol, **104**, 621-625.
12. Kumpel B. (2000) Transfusion **40**, 1376-1383.
13. Chapman JF (1999) Working party of the BCSH Blood Transfusion and General Haematology Task Forces. Transfusion Medicine **9**, 87-92.
14. Austin E. *et al*. (2009) Guidelines for the estimation of fetomaternal haemorrhage. Working party of the BCSH Transfusion Taskforce. BCSH FMH Guidelines 2009 p1-23.
15. Lloyd-Evans P. *et al*, (1999) Transfusion Medicine 9 suppl 1:33. 9.
16. Kumpel B. *et al*. (2012) Transfusion Medicine, 22 (suppl. 1), 22.
17. Belinda Kumpel *et al*. (2014 Transfusion, **54**, 1305–1316.
18. Kormoczi GF *et al* (2007) Blood 110:2148-2157.