

# REACTIVITY OF THE 'HLA-B27' MONOCLONAL ANTIBODY FD705 AGAINST 'RARE' HLA-B\*27 PRODUCTS AND ITS LIKELY EPITOPE



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### Introduction

The FITC conjugated 'HLA-B27' monoclonal antibody FD705 (catalogue number B27F50X, One Lambda) is commonly used for routine HLA-B27/B2708 typing by flow cytometry.

We have previously shown that this antibody fails to react with the products of B\*27:02 and B\*27:08 (1).

We have determined its reactivity against other B\*27 allele products and identified its likely epitope. Accordingly this has enabled us to predict its reactivity against other 'rare' B\*27 allele products.

### Materials and Methods

#### HLA-B\*27 allele products

To further determine the reactivity of FD705 against B27 specificities we tested cells from 52 subjects possessing 14 different B\*27 alleles, viz. B\*27:01 n=10, B\*27:02 n=8, B\*27:03 n=1, B\*27:04 n=2, B\*27:05 n=8, B\*27:06 n=1, B\*27:07 n=3, B\*27:08 n=5, B\*27:09 n=1, B\*27:10 n=2, B\*27:12 n=5, B\*27:14 n=2, B\*27:17 n=1 B\*27:23 n=3.

#### Flow cytometry testing

Testing used our routine flow cytometry-based B27/B2708 typing protocol (1).

### Results

#### FD705 reactivity

The FD705 monoclonal antibody gave:

**Unambiguous positive results** against B\*27:03, B\*27:04, B\*27:05, B\*27:06, B\*27:07, B\*27:09, B\*27:10, B\*27:14 and B\*27:17 specificities.

**Negative** findings against the products of B\*27:02, B\*27:08, B\*27:12 and B\*27:23.

**Variable reactions** with B\*27:01 products - some examples were positive while others were borderline positive or negative.

#### FD705 epitope

Inspection of HLA class I amino acid sequences suggested that the Bw4/Bw6-related motif **80T 81L 82L 83R** was important for FD705 reactivity.

Thus, all the B\*27 products that were clearly FD705 positive possessed this motif, while all those that were negative, with the exception of the B\*27:23 specificity, lacked the motif.

This finding with B\*27:23 suggests that other residues are involved in the FD705 epitope; a possible candidate being 70K since 70K is possessed by all FD705 positive B27 products, including B\*27:01, but is absent from B\*27:23.

Importantly, the B\*27:01 specificity, solely, has the closely related 80T 81A 82L 83R motif.

The substitution of 81L>A (with different side chain polarity and charge) may be the cause of the unpredictable reactions of B\*27:01 products with FD705 due to impaired antibody binding.

#### Predicted reactivity of FD705

On the basis of identifying the FD705 epitope as including 70K 80T 81L 82L 83R, we predict that of the 84 expressed B\*27 proteins (B\*27:01-B\*27:89) FD705 will react with 61 (72.6%).

It will be negative with 22 (26.2%) and, as seen in this study, it will be 'variable' with the B\*27:01 specificity.

### Comment

It is clearly important to be aware of an assay's ability to detect the products of 'rare' HLA-B\*27 alleles, especially when testing patients from diverse ethnic backgrounds.

### Reference

1. Coates, E. & Darke, C. (1998) Routine HLA-B27 typing by flow cytometry - differentiation of the products of HLA- B\*2702, B\*2705, and B\*2708. *European Journal of Immunogenetics* **25**, 29-38.