

Brighten up your tandem dye signals

Tandem Signal Enhancer

Tandem dyes greatly expand the range of options for multicolor flow cytometry. However, tandem dye-conjugated antibodies often show non-specific binding to cells, in particular monocytes. To overcome this drawback, Miltenyi Biotec developed the Tandem Signal Enhancer. This ready-to-use reagent increases binding specificity of tandem dye-conjugated antibodies

to cells in human PBMCs or whole blood. The addition of FcR Blocking Reagent is not required. Table 1 and figure 1 (overleaf) show the benefits of the Tandem Signal Enhancer used in combination with various tandem dye-conjugated CD20 antibodies from different providers. Background signals due to non-specific binding are reduced resulting in higher stain indices.

Conjugate	Clone	Supplier	Increase in stain index
PerCP-related tandem dyes			
CD20-PerCP-Cy™5.5	2H7	BD Biosciences	18 %
CD20-PerCP-Cy5.5	2H7	BioLegend	2%
PE-related tandem dyes			
CD20-PE-Cy7	2H7	BD Biosciences	46%
CD20-PE-Cy7	2H7	BioLegend	13 %
CD20-PE-Cy5	2H7	eBiosciences	89%
CD20-PE-Cy5.5	2H7	eBiosciences	79%
APC-related tandem dyes			
CD20-APC-Cy7	2H7	BD Biosciences	17 %
CD20-APC-Cy7	2H7	BioLegend	44%

Table 1: The Tandem Signal Enhancer increases stain indices of tandem dye-conjugated antibodies. For detailed analyses see figure 1 overleaf.

Product	Content	Order no.
Tandem Signal Enhancer	0.3 mL	130-099-887
Tandem Signal Enhancer	1 mL	130-099-888

PerCP-related tandem dyes **BD Biosciences** BioLegend 10³ 10 35.8 39.6 Relative cell number Relative cell number 46.2 36.5 10 10 10 10¹ 10² 10² 10 CD20-PerCP-Cy5.5 CD20-PerCP-Cy5.5 PE-related tandem dyes **BD Biosciences BioLegend** 10³ 10 27.4 27.7 Relative cell number Relative cell number 10 10¹ 0 0 CD20-PE-Cy7 CD20-PE-Cy7 eBioscience eBioscience 10³ 17.8 46.3 Relative cell number Relative cell number 33.6 102 10 10 10² 0 10² 10 CD20-PE-Cy5 CD20-PE-Cy5.5 **APC-related tandem dyes BD Biosciences BioLegend** 10 10 24.3 31.6 Relative cell number Relative cell number 10 10 10² 10² 10¹ 10 10 CD20-APC-Cy7 CD20-APC-Cy7

Figure 1: Immunofluorescent staining of whole blood using tandem dye–conjugated CD20 antibodies and the Tandem Signal Enhancer. Fresh lysed human whole blood was stained, in the absence or presence of the Tandem Signal Enhancer, with CD20 antibodies conjugated to various tandem dyes according to the manufacturers' instructions. Antibodies used were from BD® Biosciences, BioLegend®, or eBioscience® as indicated. Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Numbers in the dot plots indicate stain indices. Red/black numbers and lines: with/without Tandem Signal Enhancer.



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