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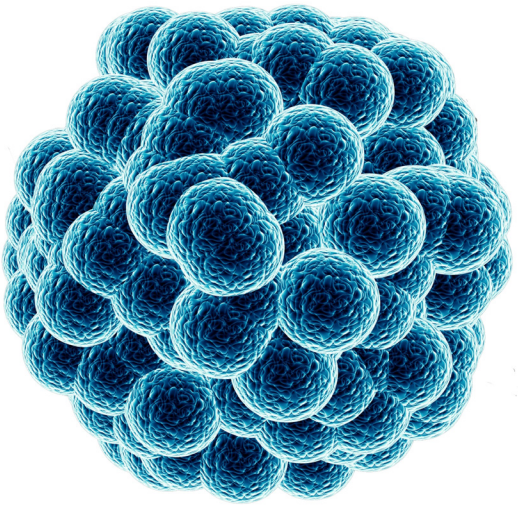

pluriSelect products in this flyer

CD19 S-pluriBead® anti-hu	11-01900-10
S-pluriBead® Mini Reagent Kit	70-50010-11

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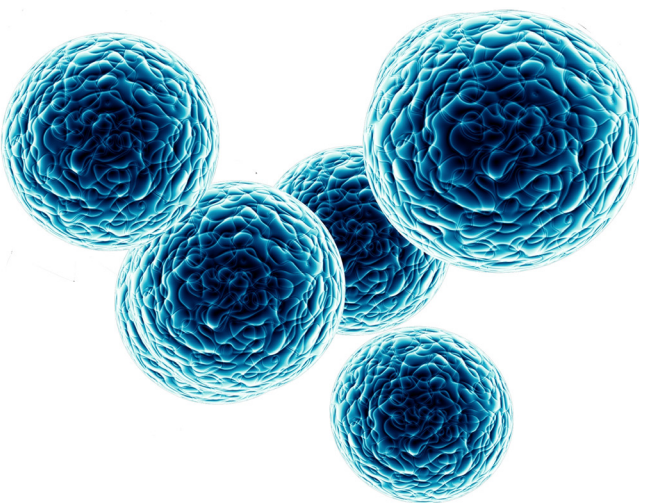
pluriBead®

Application Example

B cell isolation with pluriBead® and stimulation of antibody secretion



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B cell isolation with pluriBead® and stimulation of antibody secretion

Sample volume	50 ml whole blood (with EDTA)
Isolation method	300 µl CD19 S-pluriBead® anti-Hu
Yield	~3.5 x 10^6 B lymphocytes
Vitality	>99% (trypan blue staining)
Purity	~97% B lymphocytes
Purity determination	Anti-CD20 FITC antibody staining followed by FACS analysis

Introduction

pluriBead® allows for positive non-magnetic cell isolation from any sample material. In this example, we use CD19 S-pluriBead® for the isolation of B lymphocytes. Find the complete isolation protocol on our website or in the manual.

www.pluriselect.com/manuals-protocols.html

Frequency determination of antigen-specific memory B cells

After target activation, frequency of antibody-secreting plasma cells (ASCs) was determined via ELISpot. Isolated B cells had been stimulated for 5 days with a polyclonal activation mixture. Afterwards, they had been grown on a plate coated with the specific antigen. After 24 hours incubation, the assay had been processed.

Spots (shown in fig. 2 and fig. 3) had been counted and evaluated with a specific software (AID ELISpot6.0 iSpot). In the adjacent diagram, the blue numbers reflect the count of antigen-specific spots per well.

Results

CD19 S-pluriBead® Mini Kit allowed for isolating B lymphocytes from human whole blood sample with a purity of ~97%. The isolated targets showed a vitality of >99% after trypan blue staining. They had been successfully grown in cell culture as well as differentiated from antibody secreting plasma cells (ASCs). ASCs proved suitable for analysis of antigen-specific memory B cell response via ELISpot assay. Results had been comparable to those gained from a reference method (Dynabeads® CD19 Pan B).

For reference method, see publication: Bussmann B. M., Reiche S., Bieniek B., Krznaric I., Ackermann F., Jassoy C.: Loss of HIV-specific memory B cells as a potential mechanism for the dysfunction of the humoral immune response against HIV. Virology 2010; 397(1):7-13)

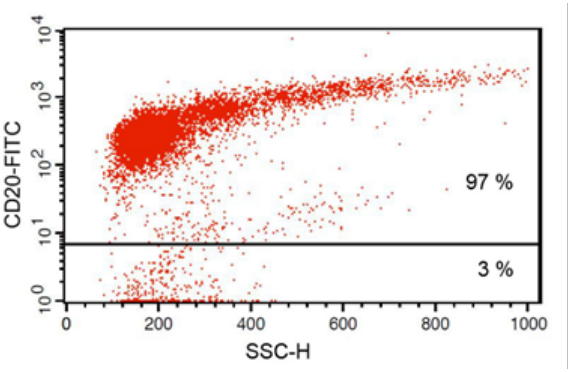

Fig. 1: Purity of isolated B lymphocytes

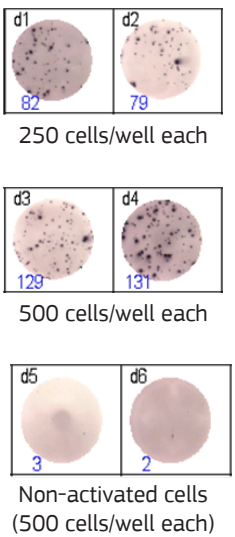

Fig. 2: ELISpot analysis of anti-Ig-specific cells in duplicates

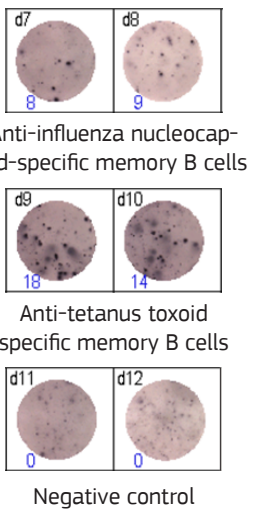

Fig. 3: ELISpot analysis of antigen-specific memory B cells in duplicates, 10^5 cells/well

Proliferation factor (Growing of B lymphocytes into ASCs after activation)	3-fold
Activation efficiency (Percentage of B lymphocytes that had been differentiated into ASCs)	32.2%
Influenza nucleocapsid-specific memory B cells	0.03%
Tetanus toxin-specific memory B cells	0.05%

Table 1: ELISpot analysis of B cell