



PPAb response

protocol type: experiment @

Project: Plasmablast trafficking and antibody function in influenza vaccination

Laboratory: Greenberg lab

Description: To generate plasmablast-derived polyclonal antibodies (PPAb) from blood samples collected at day 7 after immunization with different influenza vaccines

Purpose: To evaluate PPAb response to different influenza vaccines

1. Document history

Date	What	Who
2/12/2013	V1.0: doc created	Xiaosong He

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3. Reagents

List all reagents used in this protocol. You MUST include the catalog number if it exists.

Name	Source	Catalog Number	Lot Number
ResetteSep Human B cell Enrichment cocktail	Stemcell Technologies	15064	

4. Major equipment and software

Name	Source	Catalog Number	Software and Version
37oC CO2 incubator	Standard lab equipment		

PROTOCOL STEPS

5. Experimental groups and controls

Examples: - Naïve201: sample#201; PBMCs in media only, no cytokine stimulation
 - Stim201: sample #201; PBMCs + IL-12

Name	Description	Control (Y/N)
All subjects	Collected from LAIV, TIV and ID TIV recipients at day 7 after vaccination	N

6. Generate PPAbs from blood samples

1. Isolate B cells from whole blood, using the ResetteSep Human B cell Enrichment cocktail (Stemcell Technologies), following the manufacturer's instruction.
2. Resuspend B cells in RPMI1640 with 10% FBS at 3 million cells/ml.
3. Culture the cells at 37oC with 5% CO2 for 7 days.
4. Collect supernatant. Stored at -70oC until being used.



7. Measuring the binding activity of PPAb to influenza vaccine by ELISA

See PPAb ELISA assay protocol (PPAbBindingPro.pdf).

8. Assays to be used for analysis

Examples: - Day 3 serum will be analyzed by ELISA for IFNg production (SOP123)
- Day 7 cells will be analyzed by FACS

Name	SOP
PPAb samples analyzed by ELISA for vaccine binding activity	