

## PHA Reconstitution

### I. Purpose

The purpose of this procedure is to reconstitute and properly store phytohaemagglutinin (PHA). PHA is an extract of *Phaseolus vulgaris* seeds, which stimulates non-specific lymphocyte expansion in cell culture. PHA is used as a positive control in ELISpot assays, and to generate non-specific activated T cell cultures, which can be used as APCs, feeder cells, in virus cultivation, etc.

### II. Reagents

Reagent	Vendor	Catalogue #
Purified Phytohaemagglutinin	Fisher	R 30852801
Sterile PBS	Sigma	D8537
Sterile 0.65mL eppendorf tubes	Fisher	07-200-186

### III. Media

R10

Reagent	Stock Concentration	Volume to Add	Final Concentration
RPMI 1640	-	500mL	-
FBS	100% (v/v)	55ml	10% (v/v)
Penicillin/Strep	Pen: 5000 IU/mL Strep: 5000ug/mL	5.5mL	Pen: 50 IU/mL Strep: 50ug/mL
L-glutamine	200mM	5.5mL	2mM
HEPES	1M	5.5ml	10mM

### IV. Storage

Lyophilized powder is stored at 2-8°C.

Reconstituted aliquots can be stored at 4°C for 3-4 weeks, or at -80°C for 6-12 months with no decrease in activity.

### V. Reconstitution

1. Take a vial of the lyophilized powder from the fridge and allow it warm to room temperature without the use of artificial heat (approximately 15 minutes).
2. Once warmed, wipe the vial with 70% ethanol, and place it in the tissue culture hood for reconstitution.

3. Add 2mL of sterile PBS to the vial. Gently mix the vial to ensure the powder is completely dissolved.
4. Add 6mL of R10 to the vial and mix well.
5. Aliquot 200uL into sterile 0.65mL eppendorf tubes, label as PHA and store in  $-80^{\circ}\text{C}$  freezer (should be about 40 tubes total).

NOTE: Since PHA could be used in the culturing of cells, it is very important to use sterile technique throughout the entire process to reduce the chance of media contamination.

NOTE: Math Overview.

Lyophilized stock is provided at 2mg. Add 8mL total of PBS and R10 for a concentration of 250ug/mL. For ELISpot, make a 1:10 dilution for a concentration of 25ug/mL. Use 10uL of the dilution per well.