


STANDARD OPERATING PROCEDURE			
	Peptide Storage, Reconstitution and Pools		SOP # PC-SOP-003
	Originated by:	Michael Baladiang	Date: 05 May 17
	Laboratory:	Processing Laboratory	Pages: 1 of 4
	Approved by:	Ildiko Toth	

I. PURPOSE:

The purpose of this procedure is to outline the procedure for storage and reconstitution of peptides at the processing laboratory, Ragon Institute.

II. SAFETY:


This protocol needs to be carried out in a biosafety cabinet in BSL 2+ Lab. Personnel are required to wear safety glasses, gloves and a disposable gown before initiating any work and a secondary pair of gloves over the first before entering the biosafety cabinet. Secondary pair of gloves MUST be removed every time while exiting from the hood even if it is for a short time.

III. REQUIREMENTS:

SUPPLIES:

Fluid-resistant disposable lab coat
 Exam gloves (two pairs when working in hood)
 Protective eyewear if desired
 Pipettors—P1000, P200, and P20 or P10
 Regular *sterile* P200 and P1000 tips
R+: 500 mL RPMI-1640
 5 mL Pen/Strep
 5 mL L-glutamine
 5 mL HEPES


Item	Manufacturer	Order Number
Sterile Aerosol Resistant Tips (P1000)	Molecular BioProducts	2279
Sterile Aerosol Resistant Tips (P200)	Molecular BioProducts	2069
Sterile Aerosol Resistant Tips (P20)	Molecular BioProducts	2149
Sterile Aerosol Resistant Tips (P10)	Molecular BioProducts	2140
RPMI-1640	Sigma	R0883
Pen/Strep (5000 IU Pen/ 5000ug/mL Strep)	Mediatech	30-001-C1
L-glutamine (200mM; 29.2 mg/mL)	Mediatech	25-002-C1
HEPES (1M; 238.3mg/mL)	Mediatech	25-060-C1
DMSO	Fisher	BP231-100
PBS	Sigma	D8537
15 mL polypropylene conicals	Falcon	352097
Nunc 1.8 mL cryovials	Nunc	375418

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IV. PROCEDURE:

Reconstitution:

1. Follow the instructions for weighing out peptides in SOP# PC-SOP-002.
2. ***IN A CLEAN CELL CULTURE HOOD:*** assemble the following:
 - a. Clean DMSO (Dimethylsulfoxide)
 - b. Clean pipettors (wipe with alcohol)
 - c. *Sterile* aerosol resistant tips in the sizes needed (usually P20, P200, and P1000)
 - d. Your peptides in the tubes you weighed them into
 - e. Clean rack(s) capable of holding whatever tubes you wish to use.
3. Place tubes containing weighed peptides in your rack. If you think it likely that there's some peptide on the outside of the tube or lid, wipe it and your gloved fingers off. Open the DMSO bottle, but leave the lid on it, loose.
4. Carefully, to avoid flying peptide powder, open the peptide tube and, using the calculated volume of DMSO necessary to make a 40 mg/mL solution, add that to the peptide tube. Close the tube and mix (shake or vortex as necessary) to dissolve the peptide. (*Warning: if you vortex too vigorously, the 1.8-mL external thread Nunc cryotubes we use for peptides may leak—there is no gasket. This is usually more of a problem with pure DMSO solutions than with aqueous ones, but beware anyway.*) If you need more than 1 mL of DMSO, ***use a fresh sterile tip every time you go into the bottle, and keep the lid on the bottle as much as possible.*** Ordinary sterile tips may be used for pipetting DMSO only; aerosol resistant tips should be used for pipetting of peptide solutions.
5. Common solution problems:
 - a. Slow solution: vortex at intervals so long as you see progress—don't give up too early, since the chunk of peptide usually dissolves eventually.
 - b. Presence of precipitate: This also sometimes clears up with time and vortexing. If repeated vortexing fails to get rid of it but the precipitate is fine and resuspends easily, simply be sure it's resuspended before you use the peptide—generally, you're diluting it so much for your final use that the precipitate will go into solution. If you are very concerned about it, follow the instructions for dealing with gel formation, below—if that doesn't work, go to the longer protocol.
 - c. Gel formation: A few peptides are very difficult to get into solution; some form a gel which doesn't really break up on vortexing. In that case, you can double the amount of DMSO being used to dissolve the peptide (i.e., add the same amount of DMSO again that you did initially, so that your final concentration is **20 mg/mL**). Most peptides will go into solution at this concentration. A few (for example, the first few Vpu Clade B OLPs) form a gel even at that concentration but can be pipetted if the tube is warmed in your hand for a bit. ***Do NOT reduce the concentration below 20 mg/mL—DMSO is too toxic to the cells.***
6. Transfer the peptide solution from the original tube into the one in which it will be stored, if necessary, using a fresh aerosol-resistant tip. If there's any chance the pipettor has come into contact with the solution, wipe it off with alcohol before proceeding to the next peptide.
7. If you are diluting the peptide at this time, take the desired amount of 40 mg/mL solution into a fresh tube and add the proper amount of diluent—usually R+, but sometimes PBS. If you

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are diluting everything in your 40 mg/mL tube, you can, of course, add your diluent to your stock tube. Mix well at room temperature—DMSO freezes at 4 Celsius.

8. Store the peptide solutions at -80 Celsius except for those dilutions you are actively using, which may be kept at 4 Celsius.

Peptide pools:

Remember that you will lose some volume in pipetting and in the mixing of different solvents (DMSO and an aqueous diluent). To compensate for this, pipet slightly more than is called for of each peptide (e.g., 16 μ L instead of 15) and add slightly more diluent than is called for (an extra 0.1 mL for a final volume of 2.5 mL, for instance).

1. Pools are usually made at a concentration of 200 μ g/mL for each peptide. Consequently, you need 5 μ L of each peptide stock (at 40 mg/mL) in the pool for each 1 mL of pool that you wish to make. If you need to use one or more peptides at 20 mg/mL, obviously, you will need 10 μ L of each 20 mg/mL peptide for each 1 mL of pool.
2. To control for the effect of the DMSO on your cells
 - a. Calculate the DMSO concentration in each pool by multiplying the number of peptides in the pool by 5; this gives you the number of μ L of DMSO/mL in that pool. Of course, if you have one or more peptides at 20 mg/mL in the pool, you need to add 5 μ L more than this for each one of them.
 - b. Multiply the μ L of DMSO/mL by the desired number of mL of pool that you want to make.
 - c. Take the pool with the largest volume of DMSO and make the total volume of DMSO of each of the other pools for this project the same—i.e., add enough DMSO to the calculated value for each pool to come to the same volume.
 - d. Then calculate how much diluent (either R+ or PBS, generally) you need to make the total come out to 1 mL, or whatever your final volume is to be.
3. Make your pools:
 - a. Into a polypropylene tube, pipet in any extra DMSO needed (as calculated in Step 2).
 - b. Pipet the desired number of μ L of each peptide (allowing a bit extra for pipetting and mixing, as noted above) in the pool into the tube, *using aerosol-resistant tips*.
 - c. When all the peptides are in the tube, add the calculated volume of diluent (allowing a bit extra for pipetting and mixing, as noted above). The usual diluent is R+, but PBS has been used.
 - d. Vortex to mix and aliquot into polypropylene tubes to freeze at -80 Celsius. If precipitate forms, just be sure the solution is well vortexed before diluting it for use—since the pools are generally used at 0.2 μ g/mL for each peptide, the dilution should take care of any solution problems.

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V. REFERENCES/ ADDITIONS/ NOTES:

Lyophilized peptides are generally very stable and can be stored at -20°C for years with little or no degradation, provided that they are kept in a cool dry place. Solubilized peptides are much more prone to degradation but several steps can be taken to minimize it (for details, see the longer protocol). To avoid degradation by bacterial or microbial proteases, peptides should be prepared in sterile solutions or sterilized by filtration after reconstitution. Use either glass or polypropylene containers—you need the chemical resistance that polystyrene doesn't have. Check the chemical resistance of the lid of the glass container if you use one. Store peptide solutions at -80 Celsius except for small aliquots that you are using frequently, which may be stored at 4 Celsius.