I. PURPOSE:

The purpose of this procedure is to outline the procedure to be carried out for peptide weighing at Ragon Institute.

II. SAFETY:

This protocol needs to be carried out in the weighing chamber in the BSL1 laboratory.

III. REQUIREMENTS:

Spatula
70% ethanol spray bottle
Kimwipes, large and small
Alcohol wipes

IV. PROCEDURE:

1) Remove peptides to be weighed from the peptide freezer and allow to warm to room temperature—this takes 30-60 minutes. Do NOT remove peptides from freezer until you are sure you will be weighing them as soon as they’ve warmed up.

2) With gloved hands and a fresh alcohol wipe (a Kimwipe wetted with 70% alcohol will also work), clean the weighing chamber—pan (gently), ring, floor, door handles, and any other surface that you think may have contaminants. Then dry (gently) with a fresh small Kimwipe. If the chamber has been left messy, do this more than once. You need to be gentle in order to avoid damage to the balance, particularly by putting too much pressure on the pan.

3) With a fresh large Kimwipe and a 70% ethanol spray bottle, clean the area around the balance and your hands.

4) Turn on the balance, if it isn’t on already, and be sure that the sliding piece below the right-hand door is slid fully toward you (forward)—if it isn’t, you’re recalibrating the balance, and your weights will both fluctuate and be inaccurate. Place a closed empty glass or polypropylene (NOT polystyrene—you need the greater chemical resistance of the polypropylene) vial on the balance (15-mL tubes do well upside down on their lids), close the doors, and re-zero it. If there is drifting, try passing the tube close to the radioactive source, turning the balance off and back on,
and checking the sliding piece mentioned above—it’s easy to knock when opening the door. Always weigh with the doors closed.

5) If you use a spatula, clean the spatula with alcohol wipes, then dry it with a fresh Kimwipe. Do this before each time you use it.

6) Remove the weighed vial from the balance, open it, and either carefully dump in some peptide or, using the spatula, carefully spoon some of the powder into the vial. It sometimes helps to pack the peptide onto the spatula by picking up some and pressing it between the spatula and the inside of the tube. It also sometimes helps to pass the stock tube near the radiation source to cut down on the static buildup; be prepared for flying bits of peptide.

7) Close the tube, wipe off the outside with a dry Kimwipe if necessary, and replace it on the balance pan.

8) Weigh the tube. If necessary, either add or subtract peptide—since the peptides vary wildly in density, it’s very hard to guess exactly what you need the first time. Try not to weigh much more than you need. However, don’t be too picky about getting exactly, say, 2.0 mg; +/- 0.1 or 0.2 mg isn’t going to be a major problem, and the more you handle the peptide, the greater the risks of contamination and loss of peptide (you can’t pick stray peptide up off the balance area or wherever else it flies, since you may also pick up contaminants).

9) Record the weight, remove the vial, and be sure the stock vial is closed as well. It’s also useful to stand up, turn away, and brush yourself off.

10) Continue on to the next peptide by starting again with Step 1.

11) When you have finished weighing your peptides, or when you have to stop and finish weighing the remainder at some other time return them to the freezer. Once you are finished with all the peptides in the rack that have been pulled for your weighing, write “done” or something similar on the label with your name on it taped to the rack and e-mail the Peptide Coordinators so that they may return the peptides to the repository.

V. REFERENCES/ ADDITIONS/ NOTES:

You must be personally trained prior to weighing out or reconstituting peptides. If you need training, contact Michael Baladiang
The biggest single consideration in handling peptides is to avoid contamination of one peptide with another. Major problems when handling dry peptides are dispersal and inaccurate weighing of the due to static charge build up on the solid peptide and the tubes. There is a small radiation source (no hazard to you) inside the weighing chamber, which helps a bit with this.

For reconstitution of peptides, see **SOP# PC-SOP-003 Peptide Storage, Reconstitution, and Pools**.