Handling of GroPep® Bioreagents IGF-I, IGF-II and IGF Analogues

General Precautions:
* Avoid peptide concentrations of less than 1 mg/ml in the absence of carrier protein
* Avoid freeze-thaw cycles

Stability / Storage:
Peptides in the lyophilized state should be stored at 2-8° C and are stable for at least 2 years, or 6 months at room temperature.

Handling Procedure:
GroPep Bioreagents IGF peptides are supplied as lyophilized preparations in glass vials in an atmosphere of nitrogen at a slight vacuum. To avoid losses care should be taken to equilibrate this vacuum when opening the vial and reconstituting the peptide.

Reconstitution and Stock Solutions:
The peptide should be reconstituted in 10 mM HCl at a concentration of 1 mg/ml. In this form it is stable for at least 3 months at -20°C or -80°C. Avoid freeze-thaw cycles.

IMPORTANT: Irrespective of size, always reconstitute the entire vial *in situ* and then aliquot. Each vial contains the stated amount of protein indicated on the label and up to 20% more by mass due to the residual moisture and counter ions retained during the freeze drying process. DO NOT estimate protein content by weight or gravimetric means as this will underestimate the actual protein content by up to 20%.

Working Solutions:
When diluting peptides to concentrations of <1 mg/ml a carrier protein should be added to minimize adsorption of the peptide to plastic or glass surfaces. A solution containing the carrier protein is best added *in situ*, immediately after reconstitution with 10 mM HCl and before removing the peptide from the vial. We recommend RIA Grade Bovine Serum Albumin (BSA) as a carrier protein at peptide concentrations of between 0.1 and 1 mg/ml, depending on individual experimental conditions. When the peptide is to be used as an additive for low-serum cell culture, foetal bovine serum (FBS) is frequently present so a carrier protein is not necessary.

Handling of 20 µg and 100 µg vials:
When reconstituting smaller quantities of peptide, a 1 mg/ml stock solution is often not practical. In these circumstances dissolve the peptide in the vial in 100 µl of 10 mM HCl and before removing from the vial, add 4 volumes of buffer solution containing 1 mg/ml RIA Grade BSA to the vial and mix. Aliquot and store at -20°C or -80°C. Avoid freeze-thaw cycles.

When the presence of a carrier protein is not desirable:
In these instances we recommend an alternative storage method. Reconstitute the peptide in the vial in 100 µl of 10 mM HCl and then aliquot the desired amounts into microfuge or screw-cap Eppendorf style centrifuge tubes, using calibrated pipettes for all steps. Immediately freeze dry, cap the tubes and store at 2-8°C in a sealed container with desiccant gel. For use, solubilize the peptide in 10 mM HCl as described above.

Sterilization:
GroPep Bioreagents products are prepared using aseptic techniques and should therefore be adequate for most purposes. If sterilization is required, the peptide solution should be filter sterilized using a membrane with low protein binding.

Care must be exercised in filtering dilute peptide solutions because of the likelihood of adsorption to the filter. Filtration is best done on either the 1 mg/ml stock solution or the 0.1 mg/ml working solution diluted in 10 mM HCl. It may be possible to filter more dilute solutions if a carrier protein is present but caution should be exercised.

Important Recommendations:
*Do not add the peptide to low protein or protein free media prior to filter sterilization.
*Use a filter membrane with low protein binding characteristics.
*Filter sterilize the IGF peptide separately at a concentration of 0.1 mg/ml (diluted in 10 mM HCl) or greater.

* NOT FOR USE IN HUMANS

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