

# Handling of GroPep Bioreagents

## Transforming Growth Factor- $\beta$ 3 (TGF- $\beta$ 3)



### General Precautions:

- \* TGF- $\beta$ 3 is soluble in buffers at pH 3.8 or below (e.g. 5 mM Citrate buffer). At neutral pH, TGF- $\beta$ 3 aggregates and tends to stick to surfaces. The protein must therefore be kept at or below pH 3.8 (1).
- \* Losses can be minimized by using low adherence plastic tips and tubes, Teflon tips and tubes or silicon coated glassware.
- \* Avoid concentrations lower than 50  $\mu$ g/ml.
- \* To use TGF- $\beta$ 3 at physiological pH, a carrier protein (e.g. RIA Grade BSA in the buffer or serum in culture medium) is required.

### Stability / Storage:

TGF- $\beta$ 3 is lyophilized from 55 mM acetic acid containing mannitol to stabilize the TGF- $\beta$ 3. The 20  $\mu$ g vials contain 2 mg mannitol and the 100  $\mu$ g vials 5 mg mannitol. The lyophilized product is best stored at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ , although it can be stored at 2 -  $6^{\circ}\text{C}$  for short periods.

### Handling Procedure:

GroPep Bioreagents TGF- $\beta$ 3 is supplied as a lyophilized preparation in mannitol in siliconized glass vials in an atmosphere of nitrogen at a slight vacuum ( $-25\text{kPa}$ ). To avoid losses, care should be taken to equilibrate this vacuum when opening the vial and reconstituting the peptide. An air-filled syringe may be introduced through the bung to equalize the pressure before opening.

**Reconstitution and Stock Solutions:** The protein should be reconstituted in acetic acid at pH  $< 3.8$  in the presence of 20% ethanol to a concentration of  $>50 \mu\text{g/ml}$ . It is best to use low adhesion tips; pre-saturate the tip with the TGF- $\beta$ 3 solution by sucking up and returning the liquid to the container and then aliquot into low adherence plastic, silicon coated, or Teflon tubes and store frozen. In this form it is stable for at least 1 month at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ . 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. Therefore DO NOT estimate protein content by weight or gravimetric means as this will underestimate the actual protein content by up to 20 %.

### Working Solutions:

Working solutions may be used for a few days if kept on ice at  $0^{\circ}\text{C}$ .

If TGF- $\beta$ 3 is required at physiological pH, ensure the medium contains a carrier protein (e.g. 0.25 % RIA Grade BSA in buffer) or serum proteins in culture medium.

(1) Pellaud J, Schote U, Arvinte T, Seelig J. (1999) *Journal of Biological Chemistry* **274**, 7699-7704.

**\* NOT FOR USE IN HUMANS**

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