

## Full Length (FL) Huntingtin (HTT) Protein Handling

Provided below are recommended handling conditions for FL HTT proteins. In general, this protein is very sensitive to temperature; monomeric HTT will form higher-ordered oligomers and/or soluble aggregates even when stored on wet ice. Soluble aggregates and higher-ordered oligomers appear within hours of a thaw and are observed on analytical HPLC SEC/MALS. Avoid exposing the protein to warm temperatures, if your experiments permit, and work quickly once thawed.

### **FL HTT HANDLING**

It is best to work with FL HTT protein solutions on wet ice. This will help slow, but not eliminate aggregation of the protein. FL HTT warmed to room temperature will quickly form soluble aggregates. When dilution is necessary, dilute immediately prior to use. Never vigorously agitate the tube—this will result in the formation of bubbles as the formulation contains detergent. As a general rule, bubbles lead to protein oxidation and otherwise contribute to protein instability.

FL HTT proteins are supplied in Eppendorf LoBind micro-centrifuge tubes or equivalent. Quickly spin down the sample by centrifugation or manually flick the sample at time of thaw to recover material trapped in the lid or on the sides of the tube. The preferred method of mixing thawed protein solution is to gently mix with a micro-pipettor using a polypropylene tip. (Do not use larger pipettors or polystyrene pipette tips.) Be mindful as polystyrene tips and tubes will bind HTT protein resulting in sample loss. Note, the current HTT formulation is free of serum and albumin.

### **FREEZE/THAW OF PROTEIN**

Usually a quick freeze and a quick thaw are the best methods for retaining monomeric FL HTT. Never freeze and thaw the protein solution at  $-20^{\circ}\text{C}$ . Flash freeze remaining sample in liquid nitrogen and then store at  $-70^{\circ}\text{C}$  or below. Rapid freezing and thawing prevents phase partitioning of the salt and protein. If the HTT solution is found to be cloudy upon thawing, the oligomeric state of the protein is likely to have been adversely affected, i.e., higher-ordered oligomers and/or aggregates have formed. We have found up to 6 total quick freeze/thaw cycles do not cause significant oligomerization or aggregation of FL HTT. Long-term stability of FL HTT has been observed up 24 months without a significant change in oligomeric state.