

Caroline J. DeHart and Luca Fornelli

❖ Reagent and Materials List

Item	Part Number	Vendor
Ubiquitin	U6253	Sigma Aldrich
Trypsinogen	T1143	Sigma Aldrich
Myoglobin	M5696	Sigma Aldrich
Carbonic Anhydrase	C2624	Sigma Aldrich
Optima Grade Water	W6	Fisher Scientific
Optima Grade Acetonitrile	A955	Fisher Scientific
MS-Grade Formic Acid	PI-28905	Fisher Scientific
1.5 mL Protein LoBind Microcentrifuge Tubes	13-698-794	Fisher Scientific

❖ Important Notes

- ◆ Use 1.5 mL Eppendorf LoBind microcentrifuge tubes for protein stock preparation, top-down (TD) standard preparation, and long term aliquot storage. In our experience, these tubes have shown the lowest degree of plasticizer leaching and/or protein binding during use and storage.
- ◆ Approximate final protein amounts (loaded on-column): 0.1 pmol ubiquitin, 0.5 pmol trypsinogen, 1 pmol myoglobin, and 0.6 pmol carbonic anhydrase. Superoxide dismutase (SOD1) is present as a contaminant in carbonic anhydrase.
- ◆ A TD standard prepared in this way should be stable for up to three days at 4 °C (before significant protein oxidation becomes evident).

❖ Recipe

- ◆ Prepare 2 mg/mL stocks of each protein standard in Optima H₂O. (Aliquots can be stored at -80 °C.)
- ◆ Prepare the following (volumes shown from respective stock solutions):

Protein	Volume (μL)	Stock Concentration (pmol/μL)	Amount Loaded on Column (pmol, 1X)
Carbonic Anhydrase	40	25.7	0.64
Myoglobin	40	43.9	1.09
Trypsinogen	25	19.6	0.49
Ubiquitin	2.5	5.5	0.14
Total	107.5		

- ◆ Divide final mixture into 2.5 uL aliquots and store at -80 °C.

❖ Preparation

- ◆ Dilute one aliquot of TD STD in 100x vol. of Buffer A (95% Optima H₂O, 5 % Optima Acetonitrile, 0.2% MS-grade formic acid), where 1x vol. is the intended injection volume (e.g. **600 μL Buffer A** for an intended injection volume of **6 μL**). This will ensure that the correct amount of each TD standard protein is present in each injection.
- ◆ Mix thoroughly by pipetting, then transfer to a clean autosampler vial. The standard is now ready for use.