

Buffer Exchange and Concentration Adjustment Protocol

1. Prepare HT buffer and stock buffer.

Components	HT Buffer (at least 50 mL)		Stock Buffer (at least 10 mL)	
	Concentration		Concentration	
HEPES	50	mM	50	mM
Magnesium chloride	10	mM	10	mM
Potassium chloride	100	mM	100	mM
Glycerol	-	-	60%	(v/v)
TCEP*	1	mM	1	mM

* Add just before use

2. Dilute the eluate with 25 mL of HT buffer and keep the mixture on ice.
3. Add 15 mL of the diluted eluate to a 15 mL 3 kDa cutoff centrifugal filter and concentrate to a volume of 1.5 mL.

NOTE: One round of exchange/concentration takes about 60 min spinning at 4000 x g at 4 °C.

4. Add the remaining 15 mL of diluted eluate to the filter with the concentrated solution and concentrate to 1.5 mL once more.
5. Add 10 mL of HT buffer to the concentrated sample and concentrate to a final volume of 1 mL.
6. Transfer the concentrated sample to a 2 mL microtube.
7. Add an equal amount of stock buffer to the concentrated sample.
8. Measure the protein concentration using a NanoDrop at 280 nm.

NOTE: Prepare the blank by mixing 1 ml of HT buffer with 1 ml of stock buffer.

9. Concentrate the sample with a 0.5 mL 3 kDa cutoff centrifugal filter to reach a final protein concentration of 20 mg/mL (at 14000 x g at 4 °C).