



Test Samples available for the 2026 short course

Human Hemoglobin (Hgb) [Sigma: H7379] Assuming $A_{280}(1 \text{ mg/mL}) = 1$ (calculated is ~ 0.8).

~ 64 kDa heterotetramer ($\alpha_2\beta_2$) | Buffer: 50 mM TrisHCl pH 8.0

0.8 mg/mL (12 μM)

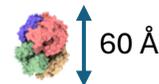
0.4 mg/mL (6.25 μM)

Some blot parameters that have worked before:

400 mesh R1.2/1.3 Cu Quantifoil

3.5 μL applied to glow-discharged grids

Leica EM-GP set at 4°C and ~ 70 -80% humidity. Wait time: 5 s. Blot time 3-5 s.



β -Amylase from sweet potato [Sigma: A8781] Using $A_{280}(1 \text{ mg/mL}) = 1.76$ (calculated)

~ 224 kDa homotetramer (56 kDa monomer) | Buffer: 20 mM HEPES pH 7.4, 50 mM NaCl

0.8 mg/mL (3.6 μM)

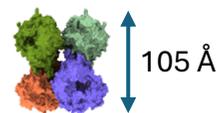
0.4 mg/mL (1.8 μM)

Some blot parameters that have worked before for 0.4 mg/mL:

300 mesh R1.2/1.3 Cu UltrAUFoil

3.4 μL applied to glow-discharged grids

Leica EM-GP2 set at 10°C and 95% humidity. No wait time. Blot time 3-4 s.



Rabbit 60S/80S ribosomes (mostly 60S) [Gifted by Dr. Brian Wimberly] $\epsilon_{260} = 40.5 \times 10^6 \text{ M}^{-1}\text{cm}^{-1}$

~ 3 MDa (60S) to ~ 4.5 MDa (80S): multicomponent protein:RNA complex

Buffer: 20 mM HEPES-KOH pH 7.6, 150 mM K-acetate, 2 mM Mg-acetate, 1 mM DTT

~ 1.5 -2.0 mg/mL (0.490 μM)

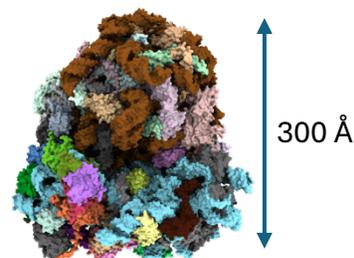
Some blot parameters that have worked before:

300 mesh R1.2/1.3 Cu Quantifoil

3.0 μL applied to glow-discharged grids

Vitrobot set at 4°C and ~ 90 -100% humidity. No wait time. Blot time 2-3 s (shorter is better because you need thicker ice with a larger particle).

Ribosomes like to stick to carbon, so a double blot might be interesting.



Grids: Carbon foil grids only!! But you can try C-flat vs. Quantifoil, different mesh sizes, different hole sizes. Double blot. Be creative!