Human Serum Albumin SAXS Standard Kit for BioSAXS Measurement

Item: 1019893

Purpose: To prepare HSA protein for measurement.

Materials required:

Rigaku HSA standard kit, micro-centrifuge, 1.5 ml tubes

[*Note: The Rigaku HSA standard kit includes ~10 mg of HSA and 5-10 ml of HSA buffer (50 mM phosphate buffer pH 6.5, 50 mM NaCl)].

Method:

1. Carefully remove the parafilm from the lids of both tubes (store at 4˚C when not in use).
2. Using a micro-centrifuge, spin the HSA solid at 10,000 x *g* for 1 min.
3. Suspend the HSA in 0.5 ml of chilled HSA buffer by carefully pipetting up and down until completely dissolved.
4. Centrifuge the HSA sample in a micro-centrifuge at 15,000 x *g* for 10 min at 4˚C.
5. Serially dilute the ~20 mg/ml* HSA stock solution as indicated below. Keep the dilutions at 4˚C until use.
    a. 100 µl 20 mg/ml HSA + 100 µl of HSA buffer = 200 µl of 10 mg/ml HSA
    b. 100 µl 10 mg/ml HSA + 100 µl of HSA buffer = 200 µl of 5 mg/ml HSA
    c. 100 µl 5 mg/ml HSA + 100 µl of HSA buffer = 200 µl of 2.5 mg/ml HSA
    d. 100 µl 2.5 mg/ml HSA + 100 µl of HSA buffer = 200 µl of 1.25 mg/ml HSA

[*Note: A more accurate determination of protein concentration should be made by an A_{280} measurement. The extinction coefficient for HSA at 280 nm is 35,700 M^{-1} cm^{-1} (Leggio et al. (2008) *Phys. Chem. Chem. Phys.* 10, 6741-6750). Ultimately, you can multiply the final dilution-corrected A_{280} by 1.88 mg/ml].

6. Collect SAXS data for a.) – d.) above.

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