Deproteinisation

In some cases it may be possible to test samples directly with appropriate dilution in distilled water. However if this is not adequate then deproteinisation with either perchloric acid, TCA or carrez reagents may be required (see methods below)**. It is assumed that the sample being tested contains the analyte at a concentration above the limit of detection of the test kit being used after accounting for the dilution during sample preparation.**

Option 1: Deproteinisation with perchloric acid:

1 M Perchloric acid:
(Sigma Cat No. 244252; MW 100.46; d = 1.664 (g/mL); 70% assay; 11.59 M)
Add 8.6mL perchloric acid to 92.4 mL of distilled water and mix thoroughly.

1 M potassium hydroxide:
(Sigma Cat No. 60369; MW 56.11; 86% assay)
Add 6.5 g of potassium hydroxide pellets to approximately 80 mL of distilled water and stir to dissolve. Make to 100 mL with distilled water.

Deproteinise samples containing protein by adding an equal volume of ice-cold 1 M perchloric acid with mixing. Filter or centrifuge at 1,500 g for 10 min and adjust the pH of the supernatant to between 7 and 8 with 1 M KOH. Use the supernatant in the assay after appropriate dilution. Alternatively, use trichloroacetic acid.

Option 2: Deproteinisation with trichloroacetic acid:

50 % (w/v) trichloroacetic acid (approx. 3 M):
(Sigma Cat No. 33731; MW 163.39)
Add 50 g of trichloroacetic acid to approximately 80 mL of distilled water and stir to dissolve. Make to 100 mL with distilled water.

Deproteinise samples containing protein by adding an equal volume of ice-cold 50 % (w/v) trichloroacetic acid with mixing. Filter or centrifuge at 1,500 g for 10 min and adjust the pH of the supernatant to between 7 and 8 with 1 M KOH. Use the supernatant in the assay after appropriate dilution. Alternatively, use perchloric acid.

***Note**:* The final trichloroacetic acid concentration given in the procedure above is 25 % (w/v). For samples with lower protein contents the final trichloroacetic acid concentration may be reduce to as low as 10 % (w/v) by altering the ratio of trichloroacetic acid and sample appropriately.

Option 3: Deproteinisation with carrez reagents

Concentrated Carrez I solution: 200 mL
Dissolve 30 g of potassium hexacyanoferrate (II) {$K_4[Fe(CN)_6.3H_2O$}
(Sigma cat. no. P-9387) in 200 mL of distilled water. Store at room temperature.

Concentrated Carrez II solution: 200 mL
Dissolve 60 g of zinc sulphate {$ZnSO_4.7H_2O$} (Sigma cat. no. Z-4750) in
200 mL of distilled water. Store at room temperature. (Sigma Cat No. 244252; MW 100.46; d = 1.664 (g/mL); 70% assay; 11.59 M)

Procedure:
Heat biological samples at approx. 80°C for 20 min then centrifuge at 13000 x g for 10 min and recover the supernatant. Add Carrez Reagent II at 1:100 volume of the supernatant and vortex to mix, then add Carrez Reagent I at 1:100 volume of the supernatant and vortex to

mix. Centrifuge the sample again at 13000 x g for 10 min a recover the clarified supernatant for use in the assay. If necessary dilute the supernatant for assay.

Note: the final volume of the clarified supernatant will be approximately one quarter of the starting volume of the original sample. Therefore adjust the volume of the staring material as required to obtain sufficient volume of clarified sample for the test.