FT-R5594A

Protein Preparation Reagent
Compatible for protein assays

Product Description

Protein Preparation Reagent (PPR) **R5594A, 500mL**

Contains: Reagent 1, 250mL
Reagent 2, 250mL

This reagent dispose of non-protein substances in your sample by selectively precipitating the protein. It is useful upstream to many analysis in order to desalt proteins or remove interfering compound, in particular for BC Assay, Coo Assay and Lavapep protein assays.

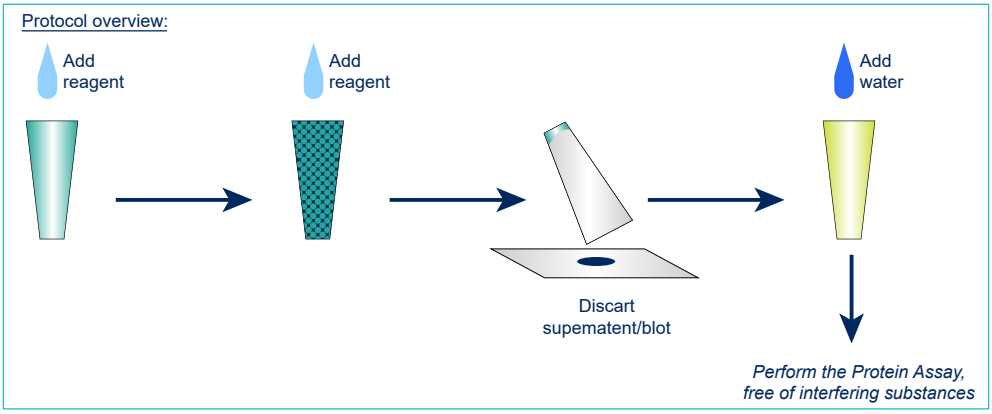
To be used with:	Product Name and Description
#UP40840A	BC Assay Protein Quantitation Kit - protein assays based on bicinchoninic acid
#UPF8640A	Coo Assay Protein Quantitation - protein assays based on Bradford method (coomassie)
#CH4191	Lavapep peptide and protein assay - protein assays based on epicocconone (fluorescent)

Storage: On receipt store at room temperature (18 months) (Z) (keep BSA at + 4°C for long term storage)
For laboratory use only, not for drug, household or medical use

Storage:
at room temperature (Z)

Features:
Precipitates proteins out of solution, leaving potentially interfering substances to be decanted away without dialysis or gel filtration, saving time and avoiding sample loss or dilution

- Ready-to-use reagents - room temperature-stable
- Four-steps protocol, < 10 minutes - room temperature operated
- Suitable to treat test tube and microcentrifuge tube samples amenable to very small samples (down 50µL) scalable to many samples in one round



Introduction

Buffering components, additives and a of contaminants are interfering in variety of analysis of protein samples, in particular in protein assays. Since every protein assay method differs with respect to which substances interfere, one may use the most compatible protein assay method. **Bradford assay** have good compatibility with reducers but in general mediocrous or poor compatibility with other substances. **BCA assay** have improved compatibility with most detergents, alkalis, DNA, lipids... but suffer of interferences from reducers. Interchim provides other fluorescent assays as well (**Lavapep assay**). But it may happen one have a cocktail of interfering substance for which no good assay exist. Furthermore it is generally not convenient and not accurate to choose different protein assays for different samples, as each have limitations in sensitivity, linearity and reproducibility depending on protein type.
In those cases, some sample pre-treatment to remove the interfering substances is required to make the sample incompatible with either protein assay. There are several ways to remove interfering substances, but these are generally time consuming. Dilution to decrease the concentration of the substance so that it no longer interferes is poorly helpful once initial protein concentration is no high. Dialysis may be used to remove the interfering substance, but is quite long. Alternatively, a desalting gel-filtration in an appropriate buffer or reagent is more rapid, but generally tedious and time-consuming.

The Compatible Protein Preparation Reagent allows the pre-treatment of up to 500 samples to remove interfering substances prior to total protein quantitation. After sample pre-treatment, total protein concentration can be determined using any Protein Assay, but also other analysis methods. The method consist in precipitating proteins, but unlike Acetone or TCA methods, sample preparation reagents can be stored at room temperature – no more needed to keep organic solvent in the fridge or the freezer! - and treated samples are stable on your bench top. Additionally, you will get more consistent results.

Directions for use

Test tube procedure for Sample Pre-treatment

1. Dispense 100 µl of each sample* in duplicates into a test tube.
   * samples to be analyzed, as well as protein standards and any control: It is IMPORTANT to analyze in the downstream protein assay protein standards and controls that are pre-treated in exactly the same conditions as samples. To simplify the procedure, put the right quantity of protein per tube as that that should be analyzed, rather to treat a larger quantity (see note/point 6).
2. Add 500 µl of Protein Preparation Reagent 1 to each sample tube.
   Mix each tube and incubate at room temperature for at least five minutes.
3. Add 500 µl of Protein Preparation Reagent 2 to each tube.
   Mix each tube and centrifuge at 10 000 g for at least 5 minutes.
4. Remove the supernatant by inverting each tube and blotting the open end on a clean paper toweling.
   Note: Take car to not lose the protein pellet that may form a very faint layer on the walls of the tube or even be not visible.
   Note: the tubes may be covered and stored refrigerated several days before protein analysis. However, take care of possible variations (condensation may form a liquid film, and bacterial contamination may occur).
5. Dissolve the protein pellet in the original sample volume (100µL) of ultrapure water.
   Note: Vortex vigorously to solubilize the pellet. To aid complete solubilization, the protein pellet may be dissolved in the BCA working Reagent (or the Coomassie reagent). Alternatively, the pellet can be dissolved with SDS 1-2% in water (this remains compatible with downstream BC Assay)
6. Perform the total protein assay as desired. The BC Assay is recommended for nice performances (linearity, P/P accuracy), but other colorimetric assays (Bradford) or fluorimetric may work also.
   -Use 100µL of ultrapure water for each protein assay blank tube.
   -To avoid sample transfer errors, perform the protein assay in the tube containing the dissolved protein pellet.

The PPR-treated samples can be analyzed for protein content (see next paragraph) or other purposes.

Analyzing PPR-treated Samples

The PPR-treated samples can be assayed for protein with the BCAssay for grater performances (linearity, broad working range, P/P accuracy). Other colorimetric assays (Bradford/CooAssay) and fluorimetric assays can work as well.
Pretreatment of the protein standards (BSA) usually improves the accuracy of the protein quantification. Assaying BSA standard before and after PPR-treatment also allows for assessing the PPR-treatment quality and yield.
Good removing of interfering substances using in one round of PPR pre-treatment is shown for. Good removing of interfering substances using in one round of PPR pre-treatment is shown for.

3.0M tris	5% TritonX100	20mM lysine, pH 10	1.25M sodium chloride
350mM dithiothreitol (DTT)	20mM arginine, pH 10	3.6M magnesium chloride	200mM glucose
200mM sodium acetate	4% SDS	125mM sodium citrate	200mM EDTA
20% glycerol	5% Tween20	5% ß-mercaptoethanol	1.0M imidazole, pH 10.4

*note: some of these substances/concentrations are compatible with BC Assay alone, or CooAssay alone, without PPR treatment.

Would an assay still be affected by interference after sample treatment by PPR, one may perform a additional washing of the protein pellet: use a fresh sample and perform steps 1-4 of the pre-treatment protocol, then repeat steps 2 and 3 using 500µL of Reagent 1 and 160µL of Reagent 2. followed by steps 4 and 5 before performing the protein assay.

Other Information

Related products:

BC Assay Protein Quantitation Kit #UP40840A – protein assays based on bicinchoninic acid
Coo Assay Protein Quantitation #UPF8640A, #UP36858A, #UP87542A– protein assays based on Bradford method
Lavapep peptide and protein assay #CH4191– protein assays based on epicocconone (fluorescent)
Protein TCA precipitation reagent #BI2941
For laboratory use only, not for drug, household or medical use
Any questions regarding the use of this product should be directed to Uptima.

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask: Uptima / Interchim Hotline: +33 4 70 03 73 06