NT-40840c

BC Protein Assay compatibility

Below is a list of substances and formulated buffers that are compatible with the BC Assay (ref.UP40840A), or have an acceptable compatibility, together with non-compatibility (or poor pattern). See note below for reserves on interferences and how to use the list, and the section "Dealing with Interfering substances" for guide lines to solve the problem.
Note: the list can also be used as guidelines for the MicroBC Assay (ref.UP75860A), although "compatible concentrations" have to be increased considerably (typically 4-10 fold).

Uptima BC Assay #UP40840A Compatibility

Substances: Buffers Solvents Chelates Reducers	Compatible Concentration
Buffers/detergents/Salts/Additives	*Good comp*. caution.
AA interférents avec le BCA : Try, cys, Tyr, l'acide urique	-
ACES, pH 7.8	25 mM
Acetate Na pH4.8	>200mM
N-acetylglucosamine in PBS, pH 7.2	10 mM
Ammonium sulfate	1.5 M
Aprotinin	10 mg/L
Asparagine	1 mM
Azide Na	>0.2%
Bicarbonate Na	100mM
Bicine, pH 8.4	20 mM
Bis-Tris, pH 6.5	33 mM
Borate (50 mM), pH 8.5 (#89804)	undiluted
Brij®-35	5%
Brij®-56, -58	1%
B-PER® Reagent (#586490.036.78248)	undiluted
BromoPhenol Blue in 50mM NaOH (BPB)	------
Calcium chloride in TBS, pH 7.2	10 mM
Carbonate/>Bicarbonate pH9.4	1M
Carbonate/Bicarbonate Na (0.2 M), pH 9.4	undiluted
Cesium bicarbonate	100 mM
Catecholamines	-
Cetylpyridinium chloride	n/a
Chélatants : cf section infra : la plupart interfèrent, ex EDTA, Cu2+,…	-
CHAP	7.5%
CHAPSO	5%
CHES, pH 9.0	100 mM
Choride Na	1M
Citrate pH4.8	200mM
Citrate (0.6 M), Carbonate Na (0.1 M), pH 9.0	1:8 dilution*
Citrate Na (0.6 M), MOPS (0.1 M), pH 7.5	1:8 dilution*
Cobalt chloride in TBS, pH 7.2	0.8 mM
Colorants : cf phenol red, BromoPheno Blue	-
Copper salts	------
CTAB	n/a
Creatinine	-------
Cysteine, DTE, DTT	see Reducers
DMF, DMSO … Ethanol,…	see Solvents
DOC (DeOxyCholate Na)	5%
DTAB	n/a
Dulbecco's PBS, pH 7.4 (Modified)	undiluted
EDTA, EGTA	see Chelates
Electrophoresis 2-D sample buffer [8M Urea, 4% CHAPS] [7M urea, 2M thiourea, 4% CHAPS]	undiluted
EPPS, pH 8.0	100 mM

Ferric chloride in TBS, pH 7.2	10 mM	
Forane: see TrichloroTriFluoroEthane	see Solvents	
Glucose	10 mM	
Glycerol (Fresh)	10%	
Glycine•HCl, Ph 2.8	50 mM	
Guanidine•HCl	3 M	
H_2O_2 (Hydroperoxide)	------	
HEPES, Ph 7.5	100 mM	
Hydrazides	--------	
Hydrides (Na_2BH_4 or $NaCNBH_3$)	--------	
Hydrogene peroxide (H2O2)	-	
Hydroxide na (NaOH)	100mM	
Hydrochloric Acid	100 mM	
Imidazole, Ph 7.0	50 mM	
I-PER® reagent	undiluted	
Iron salts	------	
Leammli SDS sample buffer (Electrophoresis) < [65mM Tris HCl, 10% Glycerol, 2% SDS, 0.025% BlueBromoPhenol]	------	
Leupeptin	10 mg/L	
Lipids	-	
Magnesium Chloride		
Mannitol		
M-PER® reagent	undiluted	
mem-PER® reagent	undiluted	
MES (0.1 M), NaCl (0.9%), pH 4.7	undiluted	
MES, pH 6.1	100 mM	
MOPS, pH 7.2	125 mM	
NE-PEG reagent	undiluted	
Nickel chloride in TBS, pH 7.2	10 mM	
NP40 (Nonidet)		
Octyl-beta-Glucoside	5%	
Octyl-Thio-beta-Glucoside	5%	
Oxydants : cf H2O2	-	
PBS [Phosphate (0.1 M), NaCl (0.15 M), pH 7.2 (#)]	undiluted	
Phenol Red	--------	
Phosphate Na	100mM	
PIPES, pH 6.8	100 mM	
PMFS	1mM	
PMSF (in isopropanol)	1 mM	
Potassium thiocyanate (KSCN)	3.0 M	
P-PER® reagent	------	
Réducteurs: la plupart interférents avec le BCA, cf.§ infra, ex divers carbohydrates, sucres impurs;	-	
RIPA lysis buffer [50 mM Tris, 150 mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0]	undiluted	
SDS	5%	
SDS	4.0%	
Sodium acetate, pH 4.8	200 mM	
Sodium azide	0.6%	
Sodium bicarbonate	100 mM	
Sodium bicarbonate 0.01 M, 0.14 M NaCl, 1 mM $MgCl^2$	+	article
Sodium chloride	1 M	
Sodium citrate, pH 4.8 or pH 6.4	200 mM	
Sodium Hydroxide	100 mM	
Sodium phosphate	100 mM	
Span® 20	1.0%	
Sucrose (saccharose)	40%+	
TBS [Tris (25 mM), NaCl (0.15 M), pH 7.6] (#UP914957)	undiluted	
Thimerosal	0.01%	

TLCK	0.1 mg/L
TPCK	0.1 mg/L
T-PER® reagent	1:2 dilution
Tricine, pH 8.0	25 mM
TrichloroTriFluoroEthane (Forane)	see Solvents
Triethanolamine, pH 7.8	25 mM
Tris / Tris HCl	150 mM or less
Tris (25 mM), Glycine (192 mM), pH 8.0 (#88826)	1:3 dilution*
Tris (25 mM), Glycine (192 mM), SDS (0.1%), pH 8.3 (#UP914957)	undiluted
Tris (50 mM) with 5mM $MgCl^2$	+
Triton® X-100	5.0%
Triton® X-114, -305, -405%	1.0%
Tryptophan	------
Tween®-20	5.5%
Tween®-60, -80	5%
Tyrosine	------
Urea	5 M
o-Vanadate (Na salt), in PBS, pH 7.2	1 mM
Y-PER reagent	undiluted
Zinc chloride in TBS, pH 7.2	10 mM
Zwittergent® 3-14	1.0%
Substances: Buffers Solvents Chelates Reducers	
Solvents	*Rather good comp.*
Acetone	10%
Acetonitrile	10%
DMF	10%
DMSO	15%
Ethanol	10%
Methanol	12%
TriChloroTriFluoroEthane ('forane')	compatible
Chelating agents	*Rather bad comp.*
Ascorbic acid	------
EDTA	2 mM
EGTA	--------
Sodium citrate	200 mM
Reducing & Thiol-Containing Agents	*Rather bad comp.*
Ascorbic acid	--------
Cysteine	-------
Dithioerythritol (DTE)	1 mM
Dithiothreitol (DTT)	0.6 mM
Lactose	------
Melibiose	--------
Mercaptans	------
2-Mercaptoethanol	0.01%
TCEP	-----
Thimerosal	0.01%

* Diluted with ultrapure water; ** Detergents were tested using high-purity Products, which have low peroxide content

-- Dashed-line entry indicates that the material is incompatible with the assay.

Notes regarding compatibility / interfering patterns

In most cases, interferences of substances in one protein assay (i.e. Coomassie based) can by solved choosing another one (i.e. BCAssay –or the opposite situation-). However,
- there is no universal protein assay method compatible with all sample components!
- certain combinations of substances, like reducing agent with detergents, might not find any right method, or require complementary methods to remove interfering substance (see section "Dealing with Interfering substances"):
=> a nicer solution is in most cases to combine BC Assay with the PPR reagent.

The above table lists, for conveniency to researchers, the effects of commonly used buffers and sample components. The pattern is summarized by a substance concentration that gives acceptable protein assay results (less than 10% interference on protein assay result), or a dashed line (------) for said 'incompatible' substance (written in orange). This is to use as a guide for assessing acceptable buffer components and to choose which protein assay might be most effective. But
-this **does not reflect the full pattern**, i.e. the concentration-dependent pattern, nor if the substance affects - increase or decrease - the background or the signal. I.e.
blue dyes typically just increase the background that reduce the dynamic range of the assay (and linearity);
Detergents change the solubility (i.e. Coomassie-protein binding) and reactions conditions.
Chelatants interfere with the chemical reaction (Cu2+ complexation of BC Assay), like reducers.
As reducers can do, chaotropic agents change the protein conformation, masking hydrophobic grooves, while acid/alkalis modify charged groups on proteins, hence affect Coomassie binding.
-**The effect might also change with various conditions:** depending on protein concentration, combination of substances, if the assay is performed in tubes or microplate, with different sample/reagent ratio, temperature and incubation time… In some instances, unexpected interferences can appear with only minor change of the concentration of one component in a buffer.

As a result, the researcher should validate the compatibility of the assay with his specific sample buffer and concentrations. Furthermore, he must decide if these effects are acceptable for the his specific purpose.

Dealing with Interfering substances

Following methods may eliminate or reduce effects of interfering substances in Protein Assays.

Note in any cases: for greatest accuracy, the protein standards must be treated identically to the sample(s).

• Precipitation method

Precipitate the proteins in the sample with acetone or trichloroacetic acid (TCA). The liquid containing the undesired substances, that cause interferences in protein assay, is discarded and the protein pellet is solubilized in suitable solvent/buffer or directly in the alkaline BCA Working Reagent (WR).

Limitations include:
.proteins estimation may be underestimated (bad resolubilisation)
.do no work or work with bias for some protein types (modified proteins, small ones)
.time-consuming and poor convenience in most protocols
.protein is denatured, and may even be affected (especially sensitive proteins)

A protocol detailing this procedure is available[r], but we recommend using the product Protein Preparation Reagent (PPR) #R5594A for improved performance and conveniency (easier, safe and quick procedure: 10min protocol)

• Dialysis or Gel Filtration

Remove the interfering substances by dialysis or gel filtration. This method is longer but presents advantages:
.low cost .limited effective working time (few minutes) .gentle to biomolecules
.suits virtually any type of proteins (even some that do not precipitate well)

Limitations include:
.do no work well for very short peptides

• Other methods

•**Dilution method:** Dilute the sample until the substance no longer interferes. This strategy is effective only if the starting protein concentration is sufficient to remain in the working range of the assay upon dilution.

•**Increase the amount of copper** in the Working Reagent (prepare WR as 50:2 or 50:3, Reagent A:B), which may eliminate interference by copper-chelating agents.

•change or optimize the **extraction/purification method** for your sample, that may add substances that interfers with the protein assay (detergents, reducers,…). Please inquire for Protein Extraction information[NT-Extraction] .