INSTRUCTIONS

Thermo SCIENTIFIC

Pierce Detergent Compatible Bradford Assay Kit

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23246 23246S

Number	Description
23246	**Pierce Detergent Compatible Bradford Assay Kit**, sufficient reagents for 300 test tube assays or 1500 microplate assays

Kit Contents:

Pierce Detergent Compatible Bradford Assay Reagent, 450mL, containing coomassie G-250 dye, methanol, phosphoric acid and solubilizing agents in water. Store at 4°C.

Caution: Phosphoric acid is a corrosive liquid.

Albumin Standard Ampules, 2mg/mL, 10 × 1mL ampules, containing bovine serum albumin (BSA) at 2mg/mL in a solution of 0.9% saline and 0.05% sodium azide. Store unopened ampules at room temperature. (Available separately as Product #23209)

23246S **Pierce Detergent Compatible Bradford Assay Reagent,** 25mL, sufficient reagent for approximately 15 test tube assays or 80 microplate assays

Note: Standards are not included with the sample size.

Storage: Upon receipt, store each component as indicated. Product shipped with an ice pack.

Note: Discard any reagent that shows discoloration.

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Introduction

The Thermo Scientific™ Pierce™ Detergent Compatible Bradford Assay Kit is a quick and ready-to-use modification of the well-known Bradford coomassie-binding, colorimetric method for total protein quantitation. The traditional Bradford reagent is incompatible with most detergents. The Thermo Scientific™ Pierce™ Detergent Compatible Bradford Assay Reagent contains proprietary additives that make it compatible with commonly used detergents and lysis reagents, including Triton™ X-100 and Nonidet™ P-40 (NP-40). Similar to the Bradford method, coomassie dye binds protein in an acidic medium causing an immediate shift in absorption maximum from 465nm to 595nm with a concomitant color change from green to blue. In addition, the assay is complete in just 10 minutes.

The protein assay can be performed in either test tube or microplate format. The standard working range is 100-1500µg/mL with up to 5% detergent; see Table 2 for compatible concentrations with specific detergents. Protein concentrations are estimated by reference to absorbances obtained for a series of standard protein dilutions, typically bovine serum albumin (BSA), which are assayed alongside the unknown samples. Because the color response with coomassie is nonlinear with

increasing protein concentration, a standard curve must be completed with each assay. Standards can be used directly without having to prepare them in the same detergent present in the test samples.

Important Product Information

- The Pierce Detergent Compatible Bradford Assay Reagent can be used with samples that contain or do not contain detergent.
- The Pierce Detergent Compatible Bradford Assay Reagent is green, unlike the traditional Bradford reagent, which is brown.
- Standards do NOT need to be prepared with detergent. This product has been optimized for detergent compatibility such that the difference in protein concentration estimation in standards with versus without detergent is less than or equal to 10%. For greatest accuracy, the protein standards must be treated identically to the sample(s).
- The volume of sample and reagent fills the wells in a 96-well plate to the brim. To efficiently mix the sample and reagent, pipette up and down with a pipette tip. Do not rely on a plate shaker for adequate mixing.
- Dye aggregates form in coomassie-based reagents over time. Gently mix the Pierce Detergent Compatible Bradford Assay Reagent end over end or with a stir bar to disperse the aggregates.
- Warm the Pierce Detergent Compatible Bradford Assay Reagent to room temperature before use.
- Assay incubation time is 10 minutes, but the reaction is stable for one hour.
- The micro assay (protein range = 2-25µg/mL) is compatible with 0.1% of the following detergents: Triton X-100, SDS, CHAPS, Tween™-20, Octyl β-thioglucopyranoside, and Brij™-35.

Preparation of Standards and Assay Reagent

A. Preparation of Diluted Albumim Standards (BSA)

Prepare a set of protein standards (Table 1). Dilute the contents of one Albumin Standard (BSA) Ampule into several clean vials, preferably in the same diluent as the sample(s). Note that it is NOT necessary to include detergent in the standards. Each 1mL ampule of Albumin Standard is sufficient to prepare a set of diluted standards for either working range suggested in Table 1. There will be sufficient volume for three replications of each diluted standard (two replications for the Micro Test Tube Protocol).

Table 1. Preparation of diluted Albumin Standards (BSA).

Dilution Scheme for Standard Test Tube and Microplate Protocols (Working Range = 100-1500µg/mL)			
Vial	**Volume of Diluent**	**Volume and Source of BSA**	**Final BSA Concentration**
A	0	300µL of stock	2000µg/mL
B	125µL	375µL of stock	1500µg/mL
C	325µL	325µL of stock	1000µg/mL
D	175µL	175µL of vial B dilution	750µg/mL
E	325µL	325µL of vial C dilution	500µg/mL
F	325µL	325µL of vial E dilution	250µg/mL
G	325µL	325µL of vial F dilution	125µg/mL
H	400µL	100µL of vial G dilution	25µg/mL
I	400µL	0	0 µg/mL = Blank
Dilution Scheme for Micro Test Tube or Microplate Protocols (Working Range = 2-25µg/mL)			
Vial	**Volume of Diluent**	**Volume and Source of BSA**	**Final BSA Concentration**
A	2370µL	30µL of stock	25µg/mL
B	4950µL	50µL of stock	20µg/mL
C	3970µL	30µL of stock	15µg/mL
D	2500µL	2500µL of vial B dilution	10µg/mL
E	2000µL	2000µL of vial D dilution	5µg/mL
F	1500µL	1500µL of vial E dilution	2.5µg/mL
G	5000µL	0	0µg/mL = Blank

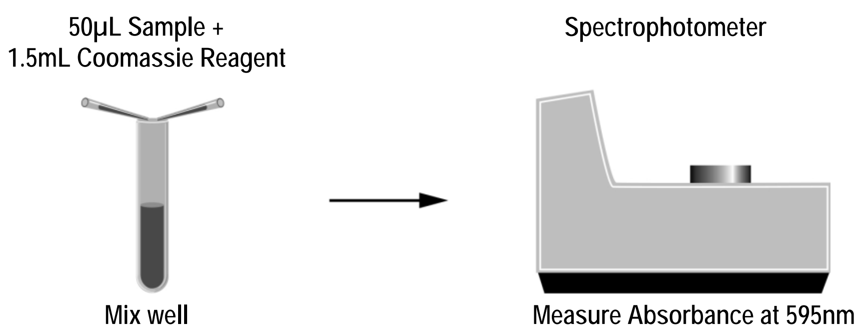
B. Equilibrating and Mixing the Pierce Detergent Compatible Bradford Assay Reagent

Mix the Pierce Detergent Compatible Bradford Assay Reagent immediately before use by gently inverting the bottle several times or stirring with a stir bar (do not shake the bottle to mix the solution). Remove the amount of reagent needed and equilibrate it to room temperature (RT) before use.

Note: Dye-dye and dye-protein aggregates tend to form in all coomassie-based reagents. If left undisturbed, the aggregates will become large enough over time to be visible. For example, when left overnight in a clear glass tube, the reagent forms dye-dye aggregates that are visible as a dark precipitate in the bottom of the tube with nearly colorless liquid above. Dye-dye aggregates can form over several hours in stored reagent, while dye-protein-dye aggregates form more quickly. Fortunately, gentle mixing completely disperses the dye-dye aggregates. Therefore, it is good practice to mix the Pierce Detergent Compatible Bradford Assay Reagent before pipetting and to mix each tube or plate immediately before measuring absorbances.

Procedure Summary

(Standard Test Tube Protocol):



Test Tube Procedures

A. Standard Test Tube Protocol (Working Range = 100-1500µg/mL)

1. Pipette 0.05mL (50µL) of each standard or unknown sample into appropriately labeled test tubes.
2. Add 1.5mL of Pierce Detergent Compatible Bradford Assay Reagent to each tube and mix well.
3. Incubate samples for 10 minutes at room temperature (RT).
4. With the spectrophotometer set to 595nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples.
5. Subtract the average 595nm measurement for the blank replicates from the 595nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average blank-corrected 595nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

B. Micro Test Tube Protocol (Working Range = 2-25µg/mL)

1. Pipette 1.0mL of each standard or unknown sample into appropriately labeled test tubes.
2. Add 1.0mL of Pierce Detergent Compatible Bradford Assay Reagent to each tube and mix well.
3. Incubate samples for 10 minutes at room temperature (RT).
4. With the spectrophotometer set to 595nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples.
5. Subtract the average 595nm measurement for the blank replicates from the 595nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average blank-corrected 595nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

Microplate Procedures

A. Standard Microplate Protocol (Working Range = 100-1500µg/mL)

1. Pipette 10µL of each standard or unknown sample into the appropriate microplate wells (e.g., Thermo Scientific™ Pierce™ 96-Well Plates, Product #15041).
2. Add 300µL of Pierce Detergent Compatible Bradford Assay Reagent to each well, preferably with a multi-channel pipettor, and pipette up and down 4-5 times to mix sample with reagent.

   Note: Use of a plate shaker for mixing is not recommended. The wells are filled to the brim, which prevents adequate mixing on a shaker.

   Note: Plate sealing tape is not necessary due to room temperature incubation, nor is it recommended because the wells are filled to the brim and sample/reagent may stick to the tape.
3. Incubate plate for 10 minutes at room temperature (RT).
4. Measure the absorbance at 595nm with a plate reader.
5. Subtract the average 595nm measurement for the blank replicates from the 595nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average blank-corrected 595nm measurement for each BSA standard vs. its concentration in µg/mL. Using the standard curve, determine the protein concentration of each unknown sample.

   Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

B. Micro Microplate Protocol (Working Range = 2-25µg/mL)

1. Pipette 150µL of each standard or unknown sample into the appropriate microplate wells.
2. Add 150µL of Pierce Detergent Compatible Bradford Assay Reagent to each well, preferably with a multi-channel pipettor, and pipette up and down 4-5 times to mix sample with reagent.

   Note: Use of a plate shaker for mixing is not recommended. The wells are filled to the brim, which prevents adequate mixing on a shaker.

   Note: Plate sealing tape is not necessary due to room temperature incubation, nor is it recommended because the wells are filled to the brim and sample/reagent may stick to the tape.
3. Incubate plate for 10 minutes at room temperature (RT).
4. Measure the absorbance at 595nm on a plate reader.
5. Subtract the average 595nm measurement for the blank replicates from the 595nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average blank-corrected 595nm measurement for each BSA standard vs. its concentration in µg/mL. Using the standard curve, determine the protein concentration estimate for each unknown sample.

   Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

Troubleshooting

Problem	Possible Cause	Solution
Absorbance of blank is acceptable, but remaining standards and samples yield lower values than expected	Improper reagent storage	Store reagent refrigerated
	Reagent still cold	Allow reagent to warm to RT
	Absorbance measured at incorrect wavelength	Measure absorbance at 595nm
Absorbances of blank and standards are acceptable, but samples yield lower values than expected	Sample protein (peptide) has a low molecular weight (e.g., less than 3000 Daltons)	Use the BCA or Lowry protein assay
A precipitate forms in all tubes	Samples not mixed well or left to stand for extended time, allowing aggregates to form with the dye	Mix samples immediately before measuring absorbances
All tubes (including blanks) are dark blue	Strong alkaline buffer raises pH of formulation, or sample volume too large, thereby raising reagent pH	Dialyze or dilute sample Remove interfering substances from sample using Product #23215
Need to read absorbances at a different wavelength	Spectrophotometer or plate reader does not have 595nm filter	Color may be read at any wavelength between 575nm and 615nm, although the slope of standard curve and overall assay sensitivity will be reduced

A. Interfering Substances

Detergents have been tested for maximum allowable percentages that are compatible with the Pierce Detergent Compatible Bradford Assay. There are several other substances that have also been tested for compatibility with this assay. Like detergents, most of these substances have only minor (tolerable) effects below a certain concentration in the original sample. Maximum compatible concentrations for many substances in the Microplate Protocol are listed in Table 3. Substances were compatible in the Standard Microplate Protocol if the error in protein concentration estimation (of BSA at 1000µg/mL) caused by the presence of the substance in the sample was less than or equal to 10%. The blank-corrected 595nm absorbance measurements (for the 1000µg/mL BSA standard + substance) were compared to the net 595nm absorbances of the 1000µg/mL BSA standard prepared in 0.9% saline.

B. Strategies for Eliminating or Minimizing the Effects of Interfering Substances

The effects of interfering substances in the Pierce Detergent Compatible Bradford Assay Reagent may be overcome by several methods.

- Remove the interfering substance by dialysis or desalting.
- Dilute the sample until the substance no longer interferes.
- Precipitate proteins with acetone or trichloroacetic acid (TCA). Upon precipitation, the liquid containing the substance that interfered is discarded and the protein pellet is solubilized in a small amount of ultrapure water or directly in the Pierce Detergent Compatible Bradford Assay Reagent. Alternatively, use Product #23215 (see Related Thermo Scientific Products).

  Note: This product has been optimized for detergent compatibility such that the difference in protein concentration estimation in standards with versus without detergent is less than or equal to 10%. For greatest accuracy, the protein standards must be treated identically to the sample(s).

Related Thermo Scientific Products

15041	**Pierce 96-Well Plates – Corner Notch,** 100/pkg
23208	**Pre-Diluted Protein Assay Standards: Bovine Serum Albumin (BSA) Set**
23212	**Bovine Gamma Globulin (BGG) Standard Ampules**, **2mg/mL**, 10 × 1mL
23213	**Pre-Diluted Protein Assay Standards: Bovine Gamma Globulin (BGG) Set**
23200	**Coomassie (Bradford) Protein Assay Kit**
23236	**Coomassie Plus (Bradford) Protein Assay Kit**
23215	**Compat-Able™ Protein Assay Preparation Reagent Set**, sufficient reagents to pre-treat 500 samples to remove interfering substances before total protein quantitation
72288	**PCC-54 Detergent Concentrate,** 3L

Additional Information

A. Please Visit the Website for Additional Information on this Product Including:

- Tech Tip #9: Quantitate Immobilized Protein
- Application Notes and more complete reference list

B. Response Characteristics for Different Proteins

Each of the commonly used total protein assay methods exhibits some degree of varying response toward different proteins. These differences relate to amino acid sequence, isoelectric point, structure and the presence of certain side chains or prosthetic groups that can dramatically alter the protein's color response. Most protein assay methods utilize BSA or immunoglobulin (IgG) as the standard against which the concentration of protein in the sample is determined. Albumin Standard Ampules (BSA) (Product #23209) provide a consistent standard for protein estimations. Nevertheless, individual proteins, including BSA and IgG, differ slightly in their color responses in the Pierce Detergent Compatible Bradford Assay (Figure 1). For greatest accuracy, the standard curve should be prepared from a pure sample of the target protein to be measured.

Table 3 shows typical protein-to-protein variation in color response. All proteins were tested at a concentration of 1000µg/mL using the Standard Microplate Protocol. The average net color response for BSA was normalized to 1.00 and the average net color response of the other proteins is expressed as a ratio to the response of BSA. The protein-to-protein variation observed with Pierce Detergent Compatible Bradford Assay Reagent is similar to that seen with other Bradford-type coomassie dye formulations.

C. Measuring Absorbances at Wavelengths Other than 595nm

If a photometer or plate reader is not available with a 595nm filter, the blue color may be measured at any wavelength between 575nm and 615nm. The maximum sensitivity of the assay occurs when the absorbance of the dye-protein complex is measured at 595nm. Measuring the absorbance at any wavelength other than 595nm will result in a lower slope for the standard curve and may increase the minimum detection level for the protocol.

D. Effect of Temperature on 595nm Absorbance

Absorbance measurements at 595nm obtained with the Pierce Detergent Compatible Bradford Assay Reagent are dependent to some extent on the temperature of the reagent. As the reagent temperature increases to room temperature, the 595nm measurements will increase. Therefore, it is important that the Coomassie reagent remain at a constant temperature (i.e., RT) during the assay.

E. Cleaning and Re-using Glassware

Use care when cleaning glassware that will be used again for protein assays. Thorough cleaning often requires the use of a detergent (e.g., PCC-54 Detergent, Product #72288), which must be completely removed in the final rinse. The coomassie dye will stain glass or quartz cuvettes. Disposable polystyrene cuvettes are a convenient alternative.

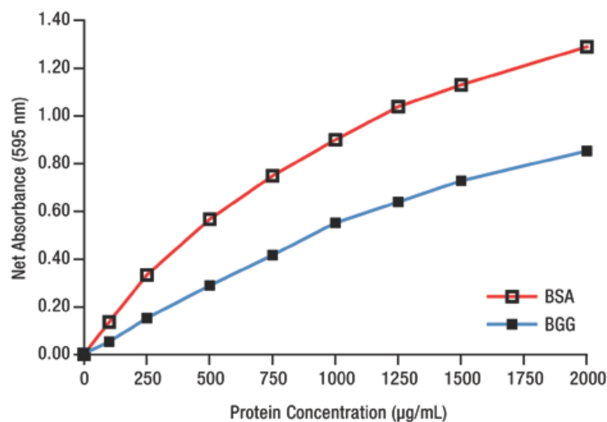


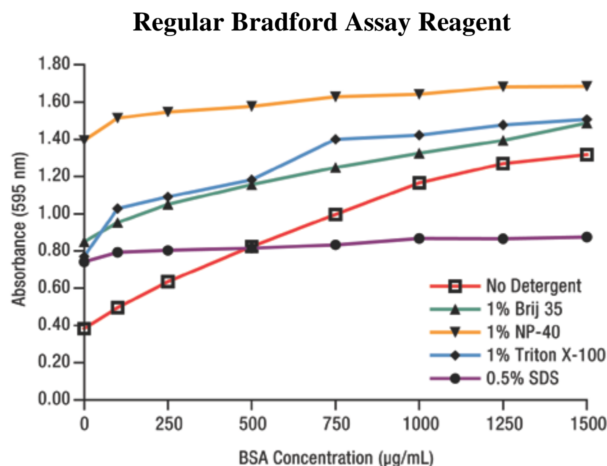
Figure 1. Typical color response curves for BSA and BGG, without detergent, using the standard microplate protocol of the Thermo Scientific Pierce Detergent Compatible Bradford Assay Kit.

Table 2. Protein-to-protein variation. Absorbance ratios (595nm) for proteins relative to BSA using the Standard Microplate Protocol in the Pierce Detergent Compatible Bradford Assay without detergent.

Ratio = (Avg "test" net Abs.) / (avg. BSA net Abs.)

Protein Tested	Ratio
Albumin, bovine serum	1.00
Aldolase, rabbit muscle	1.03
α-Chymotrypsinogen, bovine	0.43
Cytochrome C, horse heart	0.72
Gamma globulin, bovine	0.59
IgG, bovine	0.70
IgG, human	0.74
IgG, mouse	0.66
IgG, rabbit	0.31
IgG, sheep	0.59
Insulin, bovine pancreas	0.40
Myoglobin, horse heart	0.94
Ovalbumin	0.29
Transferrin, human	1.06
	0.67
Standard Deviation	**0.26**
Coefficient of Variation	**38.5%**

Regular Bradford Assay Reagent



Pierce Detergent Compatible Bradford Assay Reagent

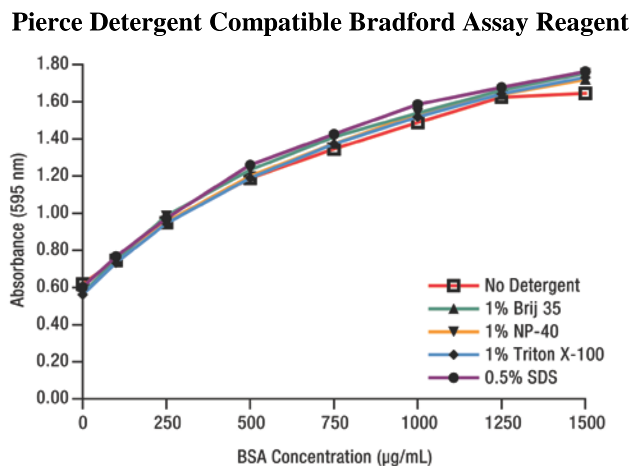


Figure 2. BSA standard curves created ± detergent using the standard microplate protocols of the Coomassie (Bradford) Assay Reagent, Product #23200 (left) and the Thermo Scientific Pierce Detergent Compatible Bradford Assay Reagent (right).

Table 3. Compatible substance concentrations in the Thermo Scientific Pierce Detergent Compatible Bradford Assay (see text for details).

Substance	Compatible Concentration
Salts/Buffers	
Ammonium sulfate	1M
Bicine, pH 8.4	100mM
Bis-Tris, pH 6.5	100mM
Borate (50mM), pH 8.5 (#28384)	undiluted
Calcium chloride in TBS, pH 7.2	10mM
Na-Carbonate/Na-Bicarbonate (0.2M), pH 9.4 (#28382)	undiluted
Glycine-HCl pH 2.8	100mM
Guanidine•HCl	1.25M
HEPES, pH 7.5	100mM
Imidazole, pH 7.0	200mM
Magnesium chloride	0.5M
MES, pH 6.1	100mM
MES (0.1M), NaCl (0.9%), pH 4.7 (#28390)	undiluted
MOPS, pH 7.2	100mM
Modified Dulbecco's PBS, pH 7.4 (#28374)	undiluted
PBS; Phosphate (0.1M), NaCl (0.15M), pH 7.2 (#28372)	undiluted
Sodium acetate, pH 4.8	180mM
Sodium azide	0.5%
Sodium bicarbonate	100mM
Sodium chloride	1.0M
Sodium citrate, pH 4.8 or pH 6.4	200mM
Sodium fluoride	5mM
Sodium phosphate	100mM
Tris	2M
TBS; Tris (25mM), NaCl (0.15M), pH 7.6 (#28376)	undiluted
Tris (25mM), Glycine (192mM), pH 8.0 (#28380)	undiluted
Tris (25mM), Glycine (192mM), SDS (0.1%), pH 8.3 (#28378)	undiluted
Chelating Agents	
EDTA	100mM
EGTA	2mM
Sodium citrate	200mM
Reducing & Thiol-containing Agents	
Ascorbic acid	50mM
L-Cysteine HCl	10mM
Dithiothreitol (DTT)	5mM
2-Mercaptoethanol	1M
TCEP	100mM

Substance	Compatible Concentration
Detergents & Cell Lysis Reagents	
Brij™-35	1.0%
Brij-58	1.0%
CHAPS, CHAPSO	5.0%
Deoxycholic acid	0.1%
Octyl β-glucoside	5.0%
Nonidet P-40 (NP-40)	1.0%
Octyl β-thioglucopyranoside	5.0%
SDS	0.5%
Triton™ X-100, X-114	1.0%
Tween™-20	1.0%
Tween-80	0.1%
RIPA lysis buffer; 50mM Tris, 150mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0 (#89900)	1/4 dilution*
M-PER™ Reagent (#78501)	Undiluted
T-PER™ Reagent (#78510)	Undiluted
Pierce IP Lysis Buffer (#87787)	Undiluted
B-PER™ Reagent (#78248)	Undiluted
B-PER Reagent PBS (#78266)	Undiluted
Mem-PER™ Plus Reagent (#89842)	Undiluted
N-PER™ Reagent (#87792)	Undiluted
NE-PER™ Reagent (#78833)	Undiluted
Y-PER™ Plus Reagent (#78999)	Undiluted
Misc. Reagents & Solvents	
Acetone	10%
Acetonitrile	10%
Aprotinin	10mg/L
DMF	10%
DMSO	10%
Ethanol	10%
Glycerol (Fresh)	10%
Hydrochloric acid	100mM
Leupeptin	10mg/L
Methanol	10%
Phenol red	0.5mg/mL
PMSF	1mM
Sodium hydroxide	75mM
Sucrose	10%
Urea	3M
o-Vanadate (sodium salt), in PBS, pH 7.2	0.5mM
Halt™ Protease Inhibitor Cocktail (#87786)	1X
Halt™ Phosphatase Inhibitor Cocktail (#78420)	1/2X
Halt™ Protease & Phosphatase Inhibitor Cocktail (#78441)	1/2X

*Diluted with ultrapure water.

General References

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