

Proteoquant™ Proteome Quantification Assay Kit USE AND STORAGE INSTRUCTIONS

INTRODUCTION

Protein Discovery's Proteoquant Proteome Quantification Assay Kit is for researchers using universal sample preparation methods to prepare whole proteome starting material for analysis. Based on a modification of the simple and scalable BCA protein quantification assay, the Proteoquant Proteome Quantification Assay Kit is compatible with the UPX™ Universal Protein Extraction Kit, the YPX™ Yeast Protein Extraction Kit, and other protocols for protein extraction into solutions containing both SDS and reducing agents.

A protocol modification, included with the kit, allows you to omit the reducing-agent compatibility step if your chosen extraction method does not utilize the reducing agents TCEP, DTT, or β-mercaptoethanol in the lysis buffer.

STORAGE AND STABILITY

Store Proteoquant Proteome Quantification Assay Kit materials at room temperature. Product shelf life is two years.

PROTOCOL 1 USAGE GUIDELINES

REDUCING AGENT COMPATIBLE ASSAY

The following usage Guidelines refer to the Proteoquant Proteome Quantification Assay Kit when it is used in accord with the Reducing Agent Compatible Assay (Protocol 1).

- The working range of the Proteoquant Proteome Quantification Assay is 125 - 2000 µg/mL.
- The Proteoquant Proteome Quantification Assay tolerates the presence of thiol reducing agents without loss of assay accuracy, up to the following concentrations:
 - TCEP: 10 mM
 - DTT: 5 mM
 - β-mercaptoethanol: 25 mM
- When SDS and one or more of the above thiol reducing agents is present, the ionic strength of the sample buffer must be maintained at or below 20 mM.
- The Proteoquant Proteome Quantification Assay is not compatible with the use of the following substances:
 - ascorbic acid
 - catecholamines
 - creatinine
 - impure glycerol
 - hydrogen peroxide
 - hydrazides
 - iron
 - some lipids
 - melibiose
 - Phenol Red
 - impure sucrose
 - tryptophan
 - tyrosine
 - uric acid

- The Proteoquant Proteome Quantification Assay Kit is compatible with the use of the following substances, but only up to the listed concentrations:

Detergents	Tween®-20	10%
	Triton® X-114	2%
	Triton X-100	7%
	CHAPS	10%
	SDS	5%
	Octyl β-thioglucopyranoside	7%
	Zwittergent® 3-14	2%
	Buffers & Salts	Tris
	HEPES, pH 7.5	200 mM
	MES, pH 6.1	100 mM
	Imidazole, pH 7.0	30 mM
	Guanidine HCl	1.5 M
	Urea	3 M
	Sucrose	40%
Chelating Agents	EDTA	5 mM
	EGTA	5 mM
	Sodium Citrate	50 mM
Other Substances	L-cysteine	2.5 mM
	Reduced glutathione	10 mM

USE AND STORAGE INSTRUCTIONS
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- If precipitates form in Reagents A or B of the Kit, redissolve by warming the bottles briefly and stirring the solutions. Do not use reagents that appear to be discolored or contaminated.
- Use accurate pipetting technique and a recently calibrated 1-10 µL pipettor for small-volume liquid transfer steps.
- Change the pipet tip for each sample when adding Compatibility Reagent.

RECOMMENDED PROCEDURE

MATERIALS NEEDED

Proteoquant Proteome Quantification Assay Kit

Clear, round-bottom, 96-well microplates

Snap cap or screw cap microfuge tube

Pipettors and disposable pipet tips

Ultrapure water

Incubator set to 37 °C

Microplate reader capable of reading absorbance at 562 nm (A_{562})

PREPARING THE CONTROLS AND STANDARDS

The Proteoquant Proteome Quantification Assay requires two controls and a serial dilution of protein standards. Prepare the following solutions:

Standard Control

Some extraction solutions such as those included Protein Discovery's UPX Universal Protein Extraction Kit and YPX Yeast Protein Extraction Kit have reducing agents pre-mixed with the lysis buffer. When this is the case, prepare a standard control composed of 200 µL ultrapure water. If the extraction solution was made up in the lab by adding one or more reducing agents to the lysis buffer prior to use, prepare a standard control composed of 200 µL of the lysis buffer used without reducing agents. The Standard Control is used to blank the spectrophotometer. The Standard Control does not contain any protein.

Sample Control

Prepare 200 µL of the same buffer used to prepare the sample, including any pre-mixed or added reducing agents. The Sample Control is used to subtract absorbance variations caused by alkylated reducing agents following sample treatment with the Compatibility Reagent. The Sample Control does not include any protein.

Protein Standards

Use the Albumin Standard to prepare a set of Protein Standards ranging in concentrations from 125-2000 µg/mL. Use ultrapure water as diluent. Diluted standards can be stored for up to six weeks at 4 °C and used for future assays.

PREPARING THE WORKING ASSAY SOLUTION

For each sample well, 260 µL of working assay solution is required. To make the working assay solution for the Reducing Agent Compatible Assay, combine Reagent A and Reagent B in the ratio of 50:1 (v:v, A:B). Prepare working assay solution daily and discard the unused portion.

PREPARING THE WORKING COMPATIBILITY REAGENT

Working Compatibility Reagent is prepared by mixing stock Compatibility Reagent with Working Reconstitution Buffer according to the directions below. Each protein sample requires 5 µL Working Compatibility Reagent.

- If the protein sample has pH>6, or does not contain EDTA or imidazole, dilute Reconstitution Buffer 1:1 with ultrapure water for use in the steps below. Otherwise use stock Reconstitution Buffer directly as Working Reconstitution Buffer.
- Use a pipette tip to puncture the foil covering of a single-use Compatibility Reagent tube.
- Add 100 µL Working Reconstitution Buffer and mix by pipetting the solution up and down 20 times to produce Working Compatibility Reagent.
- Transfer the Working Compatibility Reagent mixture to a snap cap or screw cap microfuge tube.
- Close the Working Compatibility Reagent microfuge tube and store at 4 °C protected from light. Working Compatibility Reagent can be stored in this manner for up to eight hours.
- Cut the used single-use Compatibility Reagent tube away from the unused tubes and discard it. Return the unused tubes to storage.

ASSAY PROTOCOL

1. For protein samples of pH < 5, dilute the sample 1:1 with Reconstitution Buffer.
2. Pipette 10 µL of each control, standard, or unknown sample into the center of the microplate well.
3. Add 5 µL of Compatibility Reagent to the sample in each well, using a new pipette tip for each sample.
4. Cover plate with plate sealer, and mix on plate shaker for 30 seconds.
5. Incubate for 15 minutes at 37 °C.
6. Add 260 µL of working assay solution to the sample in each well, cover plate, and mix on plate shaker for 30 seconds.
7. Incubate for 30 minutes at 37 °C.
8. Cool to room temperature (approximately 10 minutes).
9. Using the Standard Control as the blank, measure the absorbance at 562 nm on a plate reader.
10. Determine the average absorbance value of the Sample Control replicates. Subtract this average value from each of the unknown sample replicates.
11. Plot the average, blank-corrected absorbance value (A_{562}) vs. concentration (µg/mL) for each albumin standard to construct a standard curve. Determine the protein concentration of each unknown sample using the standard curve. If using a curve-fitting algorithm, select a four-parameter (quadratic) algorithm for greatest accuracy.

TROUBLESHOOTING

- Q: Why is there no color in any of the wells?
- A: Your sample may contain an unacceptable concentration of a copper chelating agent such as EDTA. You may attempt to remove the chelating agent, or, alternatively, increase the sensitivity of the assay by changing the composition of the working assay solution from 50:1 (v:v, Reagent A:Reagent B) to 50:2 (v:v, Reagent A:Reagent B).
- Q: Why is there less color than expected, even though the absorbance of my blank appears to be OK?
- A: Your sample may have changed the pH of the working assay solution. Neutralize your sample by desalting, diluting, or dialysis.
- Q: Why do all my samples, including the blank, appear to be too dark?
- A: Your sample may contain one or more of the incompatible substances listed above, or one or more of the compatible substances at an interfering concentration. If you suspect the presence of an unacceptably high concentration of a reducing agent, try diluting your sample. If you suspect the presence of catecholamines, dialyze, desalt, or dilute your sample. Interferences from lipids, if present, may be neutralized by the addition of 2% high-purity SDS detergent.

PROTOCOL 2 USAGE GUIDELINES

CONVENTIONAL ASSAY

The following usage Guidelines refer to the Proteoquant Proteome Quantification Assay Kit when it is used in accord with the Conventional Assay (Protocol 2).

- The working range of the Proteoquant Proteome Quantification Assay is 125 - 2000 µg/mL.
- The Proteoquant Proteome Quantification Assay is not compatible with the use of the following substances:
 - ascorbic acid
 - catecholamines
 - creatinine
 - cysteine
 - EGTA

- impure glycerol
- hydrogen peroxide
- hydrazides
- hydrides (Na₂BH₄ or NaCNBH₃)
- iron
- lipids
- melibiose
- Phenol Red
- impure sucrose
- tryptophan
- tyrosine
- uric acid

USE AND STORAGE INSTRUCTIONS
 Proteoquant™ Proteome Quantification Assay Kit (4 of 6)

- The Proteoquant Proteome Quantification Kit is compatible with the use of the following substances, but only up to the listed concentrations:

Detergents	Brij®-35	5%	
	Brij-56, Brij-58	1%	
	CHAPS, CHAPSO	5%	
	Deoxycholic acid	5%	
	Octyl β-glucoside	5%	
	Nonidet P-40 (NP-40)	5%	
	Octyl β-thioglucoopyranoside	5%	
	SDS	5%	
	Span® 20	1%	
	Triton® X-100	5%	
	Triton X-114, X-305, X-405	1%	
	Tween®-20, Tween-60, Tween-80	5%	
	Zwittergent® 3-14	1%	
	Buffers & Salts	ACES, pH 7.8	25 mM
		Ammonium sulfate	1.5 M
Asparagine		1 mM	
Bicine, pH 8.4		20 mM	
Bis-Tris, pH 6.5		33 mM	
Borate(50 mM), pH 8.5		un-diluted	
B-PER® Reagent		un-diluted	
Calcium chloride in TBS, pH 7.2		10 mM	
Na-Carbonate/Na-Bicarbonate (0.2 M), pH 9.4		un-diluted	
Cesium bicarbonate		100 mM	
CHES, pH 9.0		100 mM	
Na-Citrate (0.6 M), Na-Carbonate (0.1 M), pH 9.0		1:8 dilution in ultrapure water	
Na-Citrate (0.6 M), MOPS (0.1 M), pH 7.5		1:8 dilution in ultrapure water	
Cobalt chloride in TBS, pH 7.2		0.8 mM	
EPPS, pH 8.0		100 mM	
Ferric chloride in TBS, pH 7.2	10 mM		

Buffers & Salts	GlycineµHCl, pH 2.8	100 mM
	GuanidineµHCl	4 M
	HEPES, pH 7.5	100 mM
	Imidazole, pH 7.0	50 mM
	MES, pH 6.1	100 mM
	MES (0.1 M), NaCl(0.9%), pH 4.7	un-diluted
	MOPS pH 7.2	100 mM
	Modified Dulbecco's PBS, pH 7.4	un-diluted
	Nickel chloride in TBS, pH 7.2	10 mM
	PBS; Phosphate (0.1M), NaCl (0.15 M), pH 7.2	un-diluted
	PIPES, pH 6.8	100 mM
	RIPA lysis buffer; 50 mM Tris, 150 mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	un-diluted
	Sodium acetate, pH 4.8	200 mM
	Sodium azide	0.2%
	Sodium bicarbonate	100 mM
	Sodium chloride	1 M
	Sodium citrate, pH 4.8 or pH 6.4	200 mM
	Sodium phosphate	100 mM
	Tricine, pH 8.0	25 mM
	Triethanolamine, pH 7.8	25 mM
	Tris	250 mM
	TBS; Tris (25 mM), NaCl (0.15 M), pH 7.6	un-diluted
	Tris (25 mM), Glycine (192 mM), pH 8.0	1:3 dilution in ultrapure water
	Tris (25 mM), Glycine (192 mM), SDS (0.1%), pH 8.3	un-diluted
	Zinc chloride in TBS, pH 7.2	10 mM

Chelating Agents	EDTA	10 mM
	Sodium Citrate	200 mM
Reducing & Thiol-Containing Agents	N-acetylglucosamine in PBS, pH 7.2	10 mM
	Dithioerythritol (DTE)	1 mM
	Dithiothreitol (DTT)	1 mM
	Glucose	10 mM
Other Substances	β-Bercaptoethanol	0.01%
	Potassium thiocyanate	3.0 M
	Thimerosal	0.01%
	Acetone	10%
	Acetonitrile	10%
	Aprotinin	10 mg/L
	DMF	10%
	DMSO	10%
	Ethanol	10%
	Glycerol	10%
	Hydrochloric Acid	100 mM
	Leupeptin	10 mg/L
Methanol	10%	
PMSF	1 mM	
Sodium Hydroxide	100 mM	
Sucrose	40%	
TLCK	0.1 mg/L	
TPCK	0.1 mg/L	
Urea	3 M	
o-Vanadate (sodium salt), in PBS, pH 7.2	1 mM	

USE AND STORAGE INSTRUCTIONS
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Q: Why do all my samples, including the blank, appear to be too dark?

A: Your sample may contain one or more of the incompatible substances listed above, or one or more of the compatible substances at an interfering concentration. If you suspect the presence of catecholamines, dialyze, desalt, or dilute your sample. Interferences from lipids, if present, may be neutralized by the addition of 2% high-purity SDS detergent.

Q: Why did all the samples, including the blank, turn dark purple?

A: If thiols, other reducing agents, or catecholamines are present in your samples, they will turn dark purple. See the list of interfering substances above. If your protein was extracted into a buffer containing reducing agents, use Protocol 1 of this kit, instead of Protocol 2.

ORDERING INFORMATION

To order the Proteoquant Proteome Quantification Assay Kit, visit www.proteindiscovery.com to request a quote, or contact Protein Discovery, Inc. by phone, fax, or e-mail.

(T) 865.521.7400 / (TF) 866.670.9038 / (F) 865.521.3548
sales@proteindiscovery.com

DESCRIPTION

Proteoquant Proteome Quantification Assay Kit.
Contains Sufficient Reagents For 980 Microplate Assays
250 mL Reagent A, 8 mL Reagent B, ten 1 mL
tubes Albumin Standard, 48 single-use tubes Compatibility
Reagent, and 8 mL Reconstitution Buffer

PART NUMBER

44110



Protein Discovery, Inc.
418 S. Gay Street, Suite 203
Knoxville, TN 37902
www.proteindiscovery.com