Generating CB-X™ Tables and standard curves for CB-X Protein Assay Optimization

G-Biosciences

Abstract

For routine protein assays, we recommend that researchers generate their own CB-X™ Tables or standard curves. This is a one time commitment to ensure user specific results and allows single tube assays to be performed each time as opposed to generating new calibration plots for each assay. We recommend using a protein standard similar to you protein of interest or a purified source of your protein.

The supplied CB-X™ tables may result in some inconsistencies due to the type of cuvettes or microtiter plates used. CB-X™ Table for Spectrophotometer readings were measured with 1cm path length cuvettes against deionized water and CB-X™ Table for Microplate Reader readings were measured using Nunc™ Immuno 96 MicroWell™ Plates (MaxiSorp™) [Cat# 442404] against deionized water.

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Guidelines

INTRODUCTION

Protein assays are routinely used in many research fields to estimate proteins in a vast array of buffers and conditions. A major problem for researchers is to select a protein assay from the vast selection on the market that is compatible with their protein sample. CB-X™ Protein Assay eliminates this problem as it is designed to be compatible with all commonly used buffers and conditions in protein isolation, storage and assays.

For protein samples in simple, uncomplicated aqueous buffers CB-X™ is a highly sensitive, single reagent assay that can be performed in 5 minutes. CB-X™ Protein Assay uses a protein dye that is an improvement on the Bradford Coomassie dye reagent.

For complicated protein samples CB-X™ Protein Assay is supplied with reagents to clean up the samples and remove all reagents and chemicals that interfere with accurate protein estimation. These

reagents include detergents, chaotropes, reducing agents, alkylating agents, sugars, high salt concentrations, buffering agents and chelating agents (Table 1). The clean up stage and subsequent protein assay is performed in a single tube to ensure no protein loss and to maintain the accuracy of the assay.

DETERGENTS		**REDUCING AGENTS**	
Brij* 35	2%	2-mercaptoethanol	1M
CHAPS	2%	DTT	1M
CHAPSO	2%	**CHAOTROPES**	
Nonidet* P-40	2%	Guanidine.HCl	6M
SDS	2%	Urea	6M
Triton* X-100	2%	**SALTS**	
Tween* 20	2%	Ammonium sulfate	1M
Deoxycholate	0.1%	**MISCELLANEOUS**	
SUGARS		EDTA	0.1M
Glucose	1M	HEPES	0.1M
Sucrose	25%	MES	0.1M

Table 1: CB-X™ Protein Assay is compatible with many interfering agents

Figure 1 demonstrates the efficiency of the CB-X™ Protein Assay. If interfering agents are present, such as detergents, or if an artifact results are produced then the protein samples are treated with the clean up reagents and the protein is then assayed generating a linear response.

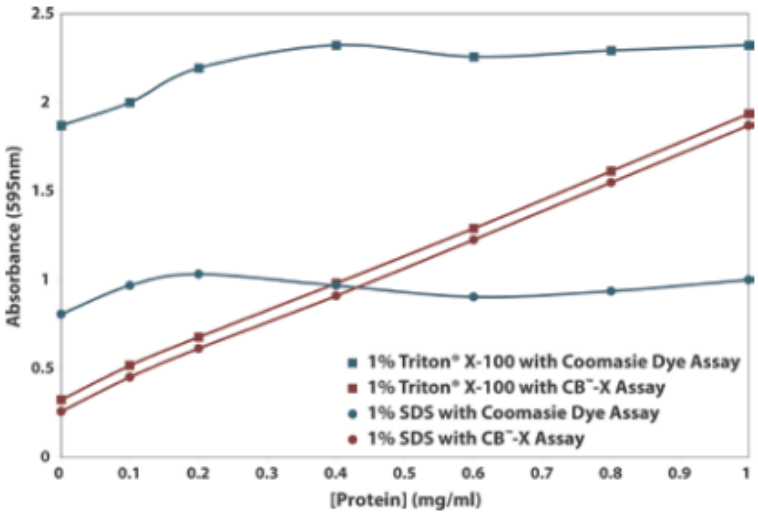

Figure 1: CB-X™ Protein Assay. Inhibitory effects of detergents on protein assays are abolished with CB-X™ . Protein solutions containing 1% Triton® X-100 or 1% SDS were assayed using a standard Coomassie dye protein assay. The same protein samples with 1% Triton® X- 100 or 1% SDS were assayed using CB-X™ protein assay. A linear response to increasing protein concentration was visualized, indicating no interference by the detergents.

CB-X™ Protein Assay is supplied with a lot specific CB-X™ Tables. These allow researchers to perform single protein clean ups, subsequent assays and then look up their absorbance in the CB-X™ Table to find the protein concentration. The CB-X™ Table eliminates the need for multiple protein standards and saves considerable time and effort. The CB-X™ Table is prepared with a complex protein mixture that compares well with proteins from mammalian, plant, bacteria and yeast sources. An optional set of bovine serum albumin standards or non-animal protein standards are supplied with Cat. # 786-12X or 786-894 respectively for generating curves when using CB-X™ Assay Dye alone or for researcher's who prefer to generate their own standard curve or to generate their own CB-X™ Table for their specific conditions.

The CB-X™ Protein Assay is reliable over the range of 0.5-50µg per assay. The regular size kit contains enough CB-X™ Assay Dye for 500 protein assays and enough clean up reagents for 250 clean ups. *(Patents Pending)*

ITEM(S) SUPPLIED

Description	Cat. # 786-11X	Cat. # 786-12X	Cat. # 786-894	Cat. # 786-12XT
CB-X™	2 x 125ml	2 x 125ml	2 x 125ml	10ml
CB-X™ Assay Dye	2 x 250ml	2 x 250ml	2 x 250ml	10ml
CB-X™ Solubilization Buffer-I	15ml	15ml	15ml	1ml
CB-X™ Solubilization Buffer-II	15ml	15ml	15ml	1ml

CB-X™ Protein Standard [2mg/ml]	-	5ml		-
Non-Animal Protein Standard [2mg/ml]	-	-	5ml	-
CB-X™ Table: Lot Specific	1	1	1	1

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival, store CB-X™ Assay Dye and protein standard at 4°C and other kit components at room temperature. The CB-X™ reagent must be chilled to -20°C for optimal efficiency, for additional convenience the CB-X™ reagent can be stored at -20°C. When stored and used properly this kit is good for 12 months.

ITEMS NEEDED AND NOT SUPPLIED WITH THIS KIT

• Centrifuge

• Assay tubes (G-Biosciences Cat # 786-008)

• Disposable polystyrène cuvettes (G-Biosciences Cat # 786-009)

PROTOCOL OPTIMIZATION

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Materials

CB-X™ Protein Assay 786-12XT by G-Biosciences

Assay Tubes (2ml) 786-008 by G-Biosciences

Disposable polystyrène cuvettes (1ml) 786-009 by G-Biosciences

Protocol

Step 1.

Prepare duplicate standards of choice in your buffer of choice at the following concentrations: 0, 0.2, 0.4, 0.6, 0.8, 1.0µg/µl

Step 2.

Transfer 50µl protein standard to 1.5ml centrifuge tubes.

Step 3.
Add 1ml pre-chilled (-20°C) CB-X™ and vortex to mix.

Step 4.

Centrifuge at 16,000xg for 5 minutes and carefully remove all the supernatant without disturbing the protein pellet.

DURATION
00:05:00

Step 5.

Add 50µl CB-X™ Solubilization Buffer-I and 50µl CB-X™ Solubilization Buffer-II to the tube and vortex to dissolve the protein pellet.

ANNOTATIONS
Colin Heath 22 Jun 2016

NOTE: Most proteins will quickly dissolve; however insoluble and membraneproteins may take 2-10 minutes of periodic vortexing.

Step 6.

Invert the CB-X™ Assay Dye 2-3 times to mix and add 1ml CB-X™ Assay Dye to the tube and vortex briefly.

Step 7.

Incubate for 5 minutes at room temperature.

DURATION
00:05:00

Step 8.

Read the absorbance at 595nm against deionized water using either a 1cm path length cuvette or transfer 200µl assay solution to a microtiter well.

Step 9.

Prepare a standard calibration plot for the determination of protein concentration of the unknown samples. Use the line equation to generate your own CB-X™ Tables.

Step 10.

This CB-X™ Table will allow all your future protein estimations to be performed without using protein standards, allowing you to carry out rapid, single protein estimations.

NOTES

Colin Heath 09 Jun 2016

NOTE: If your assay conditions change a new custom CB-X™ Table will need to be generated.