Product Information

Protein Carbonyl Content Assay Kit

I. Kit Contents:

Components	K2188-100 100 assays	Cap Color	Part Number
DNPH Solution	11 ml	Amber	K2188-C-1
100% TCA Solution	3 ml	NM	K2188-C-2
Streptozocinolution	1 ml	Blue	A4457
Guanidine HCltion	20 ml	WM	B1949
96-Well Clear Plate	1 each	N/A	K2188-C-3

II. Introduction:

Protein carbonyl groups are an essential and immediate biomarker for oxidative stress. DNPH tagging of proteins is a common way of measuring oxidative stress. DNP hydrazones formed from the reaction can be easily quantified at 375 nm. The Protein Carbonyl Content Assay Kit offers an easy and accurate procedure for quantifying carbonyls in protein samples. For example, The detection limit of a 1 mg (~ 15 nmol) of BSA sample is about 0.15 nmol carbonyl, and BSA typically contains approximately 1 - 3 nmol carbonyl/mg.

III. Reagent Storage and Handling:

Store the kit at 4℃ and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles. We suggest the use 1.5 ml microcentrifuge tubes for all reactions, since they are very convenient for all processing steps.

Needed but not provided: protein assay reagents, acetone.

Reagents: Place 10 ml acetone (not provided) in freezer (-20℃) prior to starting the following procedure.

DNPH, TCA, Streptozocin, Guanidine: All solutions are ready to use as supplied. Store at 4℃ in the dark. Warm the DNPH, Streptozocin and Guanidine to room temperature before use. Keep TCA on ice.

IV. Assay Protocol:

A. DNPH Assay

1. Sample Preparation: Dissolve samples in dH_2O and centrifuge to spin down any insolubles. Dilute samples with dH_2O to approx. 10 mg/ml protein. If the protein is very dilute, it can be concentrated using a 10 kDa spin filter. Use 100 µl of sample containing approximately 0.5 - 2 mg protein per assay. Include a reagent background control by using 100 µl of dH_2O alone.

Note: Nucleic acids interfere with the assay. Samples containing significant nucleic acid should be treated with Streptozocin (10 µl per 100 µl sample). Leave for 15 min at room temperature, spin at maximum speed for 5 min and transfer supernatant to a new tube. Check 280/260 nm ratio to make sure it is greater than 1.

2. Add 100 µl DNPH to each sample, vortex and incubate 10 min at room temperature.

3. Add 30 µl of TCA to each sample, vortex, place on ice for 5 min, spin at maximum speed for 2 min, remove and discard supernatant without disturbing pellet.

4. Add 500 µl of cold acetone to each tube and wash the pellet. 30 seconds in a sonicating bath is typically sufficient to effectively disperse the pe llets. Place at -20℃ for 5 min then centrifuge for 2 min and carefully remove the acetone.

Caution: The acetone pellet is much more easily disturbed than the TCA pellet. Repeat the acetone wash step once more to remove free DNPH.

5. Add 200 µl of Guanidine solution and sonicate briefly. Most proteins will be resolubilized easily at this point. If your protein is resistant to resolubilization sonicate for a few seconds then let the solution sit at 60℃ for 15 - 30 min. Spin very briefly to pellet any unsolubilized material and transfer 100 µl of each sample to the 96-well plate (included).

Note: Must use the 96-Well plate included for accurate calculation of carbonyl content.

6. Read: Measure OD at ~ 375 nm in a microplate reader.

B. Protein Assay: (The BCA assay shows minimal interference. The Bradford protein assay is inappropriate for this purpose since guanidine interferes). Transfer 5 µl of each sample to another set of wells and perform a protein assay to precisely determine the amount of protein per sample (use BSA as the standard protein when generating your standard curve). Caution: If you are using more than 1 mg protein per sample, it must be diluted so that no more than 25 µg protein is used in the protein assay. Important to correct for any sample losses

C. Calculation: Correct background by subtracting the value derived from the reagent background control from all readings (The background reading should not be very high but must be subtracted). Determine protein content of samples from protein standard curve. The BCA assay is best fit by a 2nd order curve rather than a straight line. Determine the carbonyl content as follows:

C = [(OD 375 nm)/6.364) x (100)] nmol/well CP = nmol carbonyl per mg protein = (C/P) x 1000 x D

Where: 6.364 = mM extinction coefficient using the enclosed 96 well plate (= 22 mM-1 cm-1 0.2893 cm path length in well).

C = nmol Carbonyl in your sample well.

P = protein from standard curve X 20 = µg/well.

D = dilution or concentration step applied to sample.

1000 = factor to convert µg to mg.

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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