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ProductInformation

GLUTATHIONE COATED HS PLATE
96-well, Clear

Product Number **P 3233**

TECHNICAL BULLETIN

Product Description

Sigma's Glutathione Coated HS 96-well Plate is designed for the capture, detection and analysis of native glutathione S-transferases (GST). A macromolecular conjugate of glutathione is non-covalently coated on the surface of the wells via a proprietary coating technology

Glutathione is coated at a reaction volume of 220 µl/well. To prevent non-specific binding, the wells are blocked with 300 µl of a bovine serum albumin solution per well.

The plate is specific for the capture of GST from crude cell lysates. Because binding occurs through the active site, this plate is selective for GST in its native conformation.

Sensitivity: Detection of ≤1 ng of GST is observed in an ELISA format with p-nitrophenyl phosphate as a substrate.

Precautions and Disclaimer

For research use only. Not for use in diagnostic procedures.

Storage/Stability

The one plate package is in a sealed bag with desiccant and a lid. The five plate package is five individually wrapped plates with desiccant and lids. For optimal performance, the unopened product should be stored in a dry place at 2-8 °C. The product may be stored at room temperature for up to three months. The product should not be exposed to temperatures above 50 °C.

Refer to the Certificate of Analysis for expiration date. The Certificate of Analysis can be obtained from the Sigma-Aldrich website (www.sigma-aldrich.com). Once opened, the product should be used promptly.

Procedure

Materials Required

Cell lysate containing GST

Glutathione Coated HS 96-well Plate	P 3233
Tris Buffered Saline w/ TWEEN® 20 (TBS-T)	T 9039
Tris buffered saline (TBS)	T 6664
Glutathione S-Transferase (*Schistosoma japonicum*; recombinant from *E. Coli*	G 5663
Rabbit Anti-GST (or other suitable primary antibody)	G 7781
Anti-Rabbit IgG (whole molecule) Alkaline Phosphatase conjugate (or other suitable secondary antibody)	A 3687
p-Nitrophenyl Phosphate (pNPP) Disodium tablets	N 2765
5 M Sodium Hydroxide (stop solution)	S 8263

Equipment Required

Multi-channel (8- or 12-channel) Pipette
Orbital shaker
Multiwell plate reader set to read absorbance at 405 nm

Standard and Sample Preparation

To estimate the GST content of a crude cell lysate, a standard curve may be generated using known amounts of GST. The log [GST] vs. signal data are fitted to a four parameter logistic curve.

1. Wash the plate three times (300 µl per well) with TBS-T. Invert the plate and gently tap out any residual liquid on paper towels.
   Note: Plate washing may be done by hand with a multi-channel pipette, or it may be done with an automated plate washer.
2. Using TBS-T as the diluent, prepare a dilution series of GST (G 5663) or other GSH affinity protein from 100 to 0.1 ng per well in 100 µl.
3. Prepare serial dilutions of the crude cell lysate with TBS-T (T 9039). The final concentration of GST should be 10 – 100 ng/well in 100 µl.
   Note: The suggested dilution range is 1:100 – 1,000 for lysates with low levels of GST and 1,000 – 10,000 for lysates with high levels of GST.

4. Apply 100 µl of TBS-T, as a blank for comparison, to no less than 4 wells.
5. Cover and incubate for one hour at room temperature (18 – 30 °C). The plate may be agitated gently with an orbital shaker during incubation.
6. Remove the plate cover and wash the plate three times (300 µl per well) with TBS-T. Invert the plate and gently tap out any residual liquid on paper towels.

Antibody Binding

Note: This section assumes use of the suggested primary and secondary antibodies. If using other primary or secondary antibodies, the appropriate dilution will have to be determined.

7. Prepare a 1:1000 dilution of the Rabbit Anti-GST (G 7781) using TBS as the diluent.
8. Apply 100 µl of this primary antibody solution per well.
9. Cover the plate and incubate for one hour at room temperature.
   Note: Proceed to step 14 if an antibody-enzyme conjugate was used in steps 7-9. If an unlabeled primary antibody was used in steps 7-9, proceed to step 10.
10. Remove the plate cover and wash the plate three times (300 µl per well) with TBS-T. Invert the plate and gently tap out any residual liquid on paper towels.
11. Prepare a 1:30,000 dilution of the Anti-Rabbit IgG (whole molecule) Alkaline Phosphatase conjugate (A 3687) secondary antibody using TBS as the diluent
12. Apply 100 µl of the secondary antibody-enzyme conjugate solution per well.
13. Cover the plate and incubate for one hour at room temperature.
14. Remove the plate cover and wash the plate three times (300 µl per well) with TBS-T. Invert the plate and gently tap out any residual liquid on paper towels.

Color Development

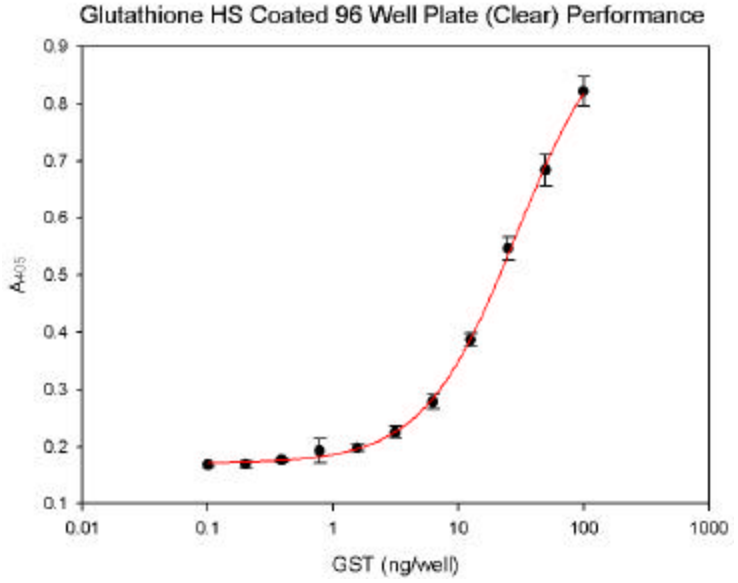
15. Prepare the substrate and stop solutions according to the product instructions. Allow them to equilibrate to room temperature.
    Note: For Sigma pNPP tablets (N 2765), dissolve 2 tablets in 20 mL TBS at pH 9.6 for every 20 mL needed (approx. 20 mL needed per plate). Sigma's 5 M sodium hydroxide (stop solution) (S 8263) is added undiluted.
16. Start the color development reaction by applying 170 µl of the p-NPP substrate per well. Time the additions precisely.
17. Incubate 45 minutes at room temperature. It may be necessary to adjust the incubation time to achieve optimal signals (absorbance at 405 nm of approximately 1 for 100 ng standards). If so, be sure that the incubation times for the samples and standards are identical.
18. Stop the color development reaction by applying 30 µl of the sodium hydroxide stop solution (S 8263) per well. Time the additions precisely so that the incubation time is identical for all of the wells.
19. Agitate the plate briefly to thoroughly mix the contents of the wells.
20. Read the absorbance of each well at 405 nm.

Calculation of Results

21. Prepare a standard curve by plotting log [GST] versus absorbance.
22. If desired, fit the standard curve data to a four parameter logistic equation.
23. Use the standard curve to determine the concentration of GST in the samples.

Results

A typical standard curve is given below:



Background readings (buffer blank) should yield absorbance readings between 0.0 – 0.2, in the absence of any interfering substances (see specificity section). A positive signal indicating the presence of GST should yield absorbance readings greater than two times the standard deviation plus the mean of the signal of background absorbance readings.

Interference

Extraction buffers may contain substances that interfere with detection and quantitation of GST. Commonly used reducing agents, detergents, and chaotropes were examined under the outlined conditions, and the results are listed below. Buffers containing phosphate should not be used with alkaline phosphatase because they may inhibit the activity of this enzyme.

Agent	Concentration	Signal ± SD (%)
None		100
Dithiothreitol	1 mM	89 ± 8
	25 mM	95 ± 9
	100 mM	93 ± 9
β-Mercaptoethanol	1 mM	83 ± 5
	25 mM	84 ± 8
	100 mM	88 ± 9
Tween-20	0.1 %	103 ± 6
	1.0 %	96 ± 9
CHAPS	0.1 %	98 ± 5
	1.0 %	71 ± 8
SDS	0.1 %	0
	1.0 %	0
CelLytic™-B	no dilution	73 ± 2
Guanidine HCl	0.5 M	27 ± 7
	1.0 M	0
Urea	1.0 M	48 ± 11
	2.0 M	0

Plate Features

Plate composition	High-binding polystyrene
Lid	Yes
Well configuration	Flat bottom/round
Well width	6.4 mm
Well depth	11 mm
Maximum recommended working volume, per well	200 µL

References

1. Simons, P.C. and Vander Jagt, D.L., Purification of glutathione S-transferases by glutathione-affinity chromatography, Methods Enzymol. 1981;77:235-7.

Troubleshooting Guide:

Problem	Possible Cause	Solution
Low/no signal from standards	Degraded standard	Repeat with fresh standard
	Antibody/Antibody-enzyme conjugate inactive	Repeat with fresh antibody/antibody-enzyme conjugate at appropriate titers. Optimize primary and/or secondary antibody if necessary
	Stop solution was added too early	Re-run the assay and optimize incubation time
	Degraded substrate or enzyme label	Mix substrate directly with the antibody-enzyme conjugate. No color indicates bad substrate or degraded conjugate. Repeat with fresh reagents
Low/no signal from samples	Sample interference	Check recovery of standard GST added to samples. If recovery is low, check for presence of interfering substances and pre-treat or dilute.
	Crude cell lysate too dilute	Reduce the dilution factor
	Degraded GST	Re-evaluate extraction to assure isolation of active GST
	No GST present	Re-evaluate samples and prepare a fresh culture for analysis
High background signal	Too much sample added	Follow recommendations for 200 µL sample size
	Cross-contamination	Keep tips for antibody-enzyme conjugate separate from tips for substrate. Do not re-use reagent trays for different substances
	Outdated or degraded plate	Store plates as recommended and use within expiration date. If improperly stored or aged beyond expiration, purchase new plates
	Degraded or contaminated substrate	Use fresh substrate
	Antibody titer too high	Optimize the antibody (indirect detection) and/or antibody-enzyme conjugate (direct detection) by increasing the dilution factor
Variable signal	Incomplete mixing	Mix the wells before reading the absorbance
	Temperature changes or gradients	Ensure all reagents and substrates are equilibrated to the recommended temperature
	Pipette error/contamination	Check pipette calibration, handle tips with clean gloves, do not draw too much solution into tips
	Imprecise timing	Time the addition of substrate and stop solution exactly
	Wells were allowed to dry during manipulations	Do not allow the wells to remain empty between steps. Make the next addition promptly
	Plate washer dispensing/aspirating improperly	Make sure the washer is functioning properly and no heads are clogged

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