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Validation Report

Report #029860 | Validated On: **05/19/16**

Summary

Antigen	Mouse anti-GST monoclonal antibody
Catalog number	ABIN3045984
Supplier	Clonegene LLC
Supplier catalog number	CG0512
Lot number	1214
Method validated	Western Blot
Laboratory	Celplor LLC
Validation number	29860
Positive Control	BL21 bacteria cells were transformed with an in-house GST expression vector pGST
Negative Control	Empty vector cell lysate
Notes	Based on the 12% GST content in total cell lysate of BL21-pGST (+IPTG), there is an estimated 1 ng of GST protein in 0.01 ug of total protein with IPTG induction. Therefore, the detection limit of mouse anti-GST monoclonal antibody is lower than 1 ng of GST protein.

Full Methods

Primary Antibody
- Antibody: Mouse anti-GST monoclonal antibody
- Supplier: Clonegene LLC
- Supplier catalog number: CG0512
- Lot number: 1214
- Dilution: 1:1000

Secondary Antibody
- Antibody: HRP labeled secondary anti-mouse antibody
- Supplier: GE Healthcare Life Sciences
- Supplier catalog number: NXA931
- Lot number: 390630
- Dilution: 1:2000

Controls
- Positive control: BL21 bacteria cells were transformed with an in-house GST expression vector pGST
- Negative control: Empty vector cell lysate

Protocol

I. Reagent preparation

BL21 bacteria cells were transformed with an in-house GST expression vector pGST.
Individual colony was inoculated in 2xYT medium and cultured at 37°C with shaking for 5 hrs followed by IPTG (1mM) induction for 2 hrs. Bacteria was grown to OD600=0.6-0.8 (5 hr) when IPTG was added.
Cells were centrifuged at top speed and pellets were collected.
Cell pellets were lysed in SDS-PAGE sample buffer.
Protein concentration was assayed using DC protein assay kit (Bio-Rad).
50 ug of cell lysate was loaded and protein was separated by SDS-PAGE.
Gel was stained with SimplyBlue Safe Stain (Invitrogen) at room temperature.
GST content in IPTG induced samples was analyzed by Quantity One software (Bio-Rad).

II. Western blot validation

Cell lysates from BL21 host cells, BL21-pGST transformed cells with or without IPTG induction were loaded and proteins were separated by SDS-PAGE followed by Western transfer to a nitrocellulose membrane.
Membrane was blocked with 2% powder milk (Bio-Rad) for 1 hr at room temperature.
Membrane was incubated with mouse anti-GST monoclonal antibody (1:1000 dilution) at 4°C overnight with shaking.
Membrane was washed with TBST three times for 10 min.
After washing, membrane was incubated with HRP labeled secondary anti-mouse antibody (1:2000 dilution, GE Healthcare) with shaking at room temperature for 1 hr.
Membrane was washed with TBST three times for 10 min.
After washing, membrane was incubated with SuperSignal West Pico substrate (Thermo Scientific) for 5 min at room temperature.
Membrane was analyzed by ChemiDoc XRS gel documentation system (Bio-Rad).

Figures

Validation Report

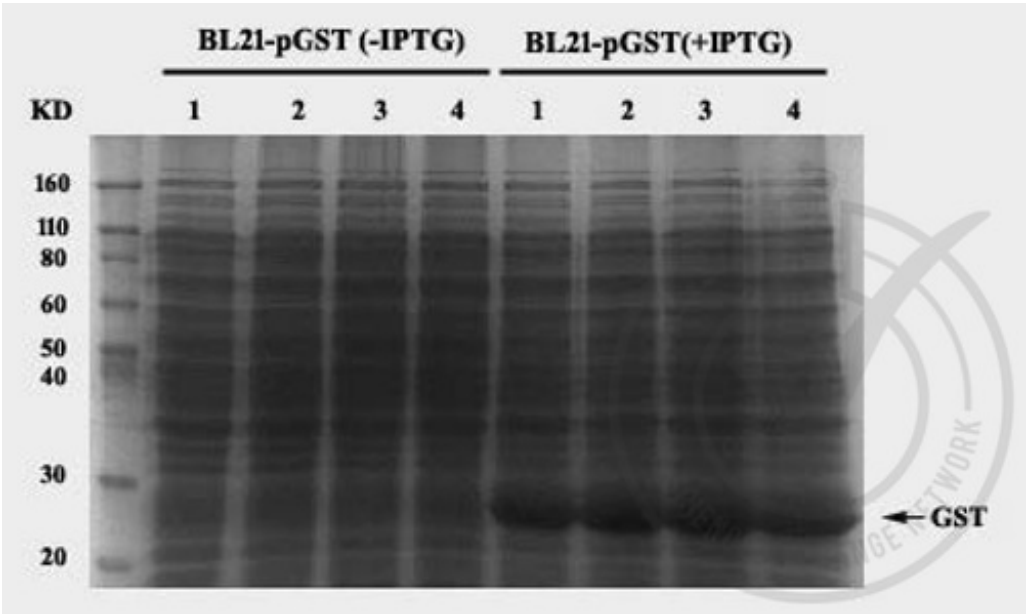

Figure 1: GST content of BL21-pGST(+IPTG) is 12% of total protein. GST content in IPTG induced samples was analyzed by Quantity One software (Bio-Rad).

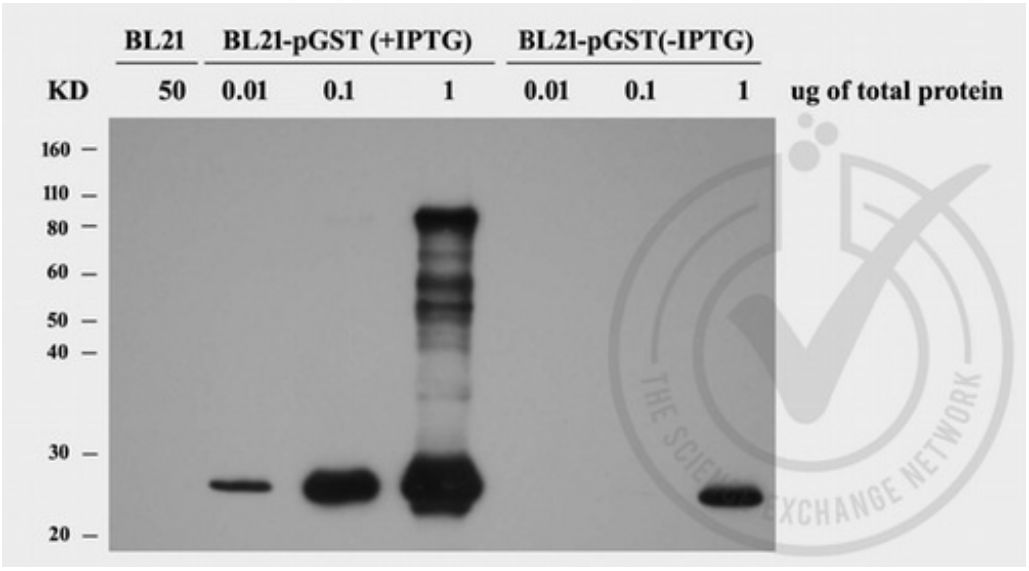

Figure 2: Western blot for mouse anti-GST monoclonal antibody. The first lane shows the empty vector cell lysate (negative control). This is followed by a 0.01, 0.1, and 1 ug of total protein from BL21-pGST, with (Lanes 2, 3, and 4) or without (Lanes 5, 6, and 7) IPTG induction, respectively. Based on the 12% GST content in total cell lysate of BL21-pGST (+IPTG), there is an estimated 1 ng of GST protein in 0.01 ug of total protein with IPTG induction. Therefore, the detection limit of mouse anti-GST monoclonal antibody is lower than 1 ng of GST protein. The higher than expected molecular weight may be the result GST dimers or aggregates (Riley et al. Protein Engineering vol.9 no.2 pp.223-23O, 1996).