Overview

Product name: GST 6XHis-tag ELISA Kit
Detection method: Colorimetric

Precision

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>8</td>
<td></td>
<td></td>
<td>6.4%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>3</td>
<td></td>
<td></td>
<td>10%</td>
</tr>
</tbody>
</table>

Sample type: Cell Lysate
Assay type: Sandwich (quantitative)
Sensitivity: > 0.5 ng/ml

Recovery

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Average %</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>75</td>
<td>66% - 90%</td>
</tr>
<tr>
<td>Cell culture extracts</td>
<td>94</td>
<td>66% - 112%</td>
</tr>
<tr>
<td>Cell culture media</td>
<td>90.1</td>
<td>78% - 106%</td>
</tr>
</tbody>
</table>

Assay duration: Multiple steps standard assay

Product overview

The 10X blocking buffer is casein based. It is normal for it to be cloudy with small precipitates in it. This does not negatively affect the assay.

The 6XHis-tag is a commonly used polyhistidine tag for the purification of recombinant proteins by immobilized metal ion affinity chromatography (IMAC) from E. coli, Saccharomyces cerevisiae, mammalian cells and baculovirus-infected Spodoptera frugiperda insect cells. It is typically used...
(1) as a single tag on proteins for purification, (2) as part of a dual tag for achieving proteins of higher purity (i.e. combined with GST fusion partner, albumin-binding protein or S-peptide) or (3) in tandem affinity purification of endogenous protein complexes.

This 6XHis-tag detection assay kit is an enzyme immunoassay developed for the sensitive detection of 6XHis-tagged fusion proteins with a suitable detector antibody or for the quantification of GST-6xHis double tagged proteins with the supplied detector antibody. The quantity of the GST 6XHis double tag can be determined in lysates or intermediate purification fractions by comparing their absorbance with that of a known recombinant GST 6XHis-tagged standard curve. The kit has a detection sensitivity limit of 0.5ng/mL of GST 6XHis-tagged protein. The kit is particularly suitable for screening in high-throughput conditions for maximum expression and optimal purification of 6XHis-tagged proteins as well as for screening humoral immunity against His-tagged antigens. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and 6XHis-tag fusion proteins.

This kit is reactive with any 6XHis-tagged recombinant protein.

Notes

Typical working ranges:

- GST His tagged: 1 – 50 ng/mL
- GSK3b His tagged: 70 – 5000 ng/mL

The 10X blocking buffer is casein based. It is normal for it to be cloudy with small precipitates in it. This does not negatively affect the assay.

Abcam has not and does not intend to apply for the REACH Authorisation of customers’ uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform

Microplate

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>1 x 96 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Blocking Buffer</td>
<td>1 x 6ml</td>
</tr>
<tr>
<td>10X GST Detector Antibody</td>
<td>1 x 700µl</td>
</tr>
<tr>
<td>10X HRP Label</td>
<td>1 x 1ml</td>
</tr>
<tr>
<td>20X Buffer</td>
<td>1 x 20ml</td>
</tr>
<tr>
<td>Anti 6XHis-tag Microplate (8 x 12 antibody coated strips)</td>
<td>1 unit</td>
</tr>
<tr>
<td>Extraction Buffer</td>
<td>1 x 15ml</td>
</tr>
<tr>
<td>GST Standard</td>
<td>1 x 2µg</td>
</tr>
</tbody>
</table>
Relevance

The H-H-H-H-H-H motif is used as a tag on many recombinant proteins to facilitate purification. The antibody recognizes the His-tag fused to the amino- or carboxy- termini of targeted proteins in transfected or transformed cells.

Cellular localization

Depends upon the localization of the parent protein tagged with hexahistidine.

Images

This kit is effective with a wide variety of sample matrices.

Other - GST 6XHis-tag ELISA Kit (ab128573)

Validation by Western blot. Capture antibody was tested by western blot against three His tagged proteins: GST recombinant protein loaded at 50ng/well (lane 1), GSK3b recombinant protein loaded at 50ng/well (lane 2) and Hexokinase 1 recombinant protein loaded at 250ng/well (lane 3). Blots were blocked with 5% Milk in TBST buffer, incubated overnight in anti 6XHis-tag primary antibody and labeled with GAM HRP.

Western blot - GST 6XHis-tag ELISA Kit (ab128573)
Example GSK3B-His tagged standard curve. GSK3B-His tagged protein (not provided with the kit) was diluted from 5µg/mL to 0.07µg/mL and loaded on to the His plate. GSK3b was detected with a rabbit monoclonal antibody and labeled with the provided HRP label. Background was subtracted from data points prior to log transformation and curve fitting (log-agonist vs. response – variable slope).

Example GST 6XHis tagged standard curve. GST-His tagged protein (provided with the kit) was diluted from 50 ng/mL to 0.7 ng/mL. Background was subtracted from data points prior to log transformation and curve fitting (log-agonist vs. response – variable slope). Sensitivity was calculated mathematically to be as low as 0.5 ng/mL.

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