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Product information:

Document reference : 62GSTPEG rev 01 (Jan 2019)

GST-tag check kit

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

Storage temperature : 2-8°C

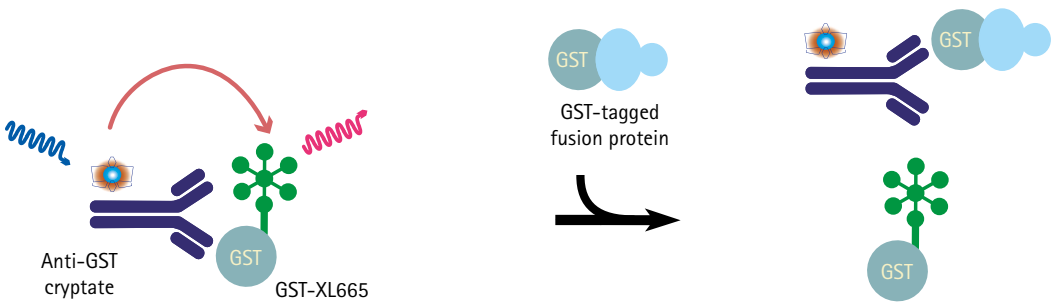
Packaging details :

	384-well low volume plate (20 µL)
62GSTPEG	500 tests

1. Assay description and intended use

This kit enables the rapid detection of GST-tagged fusion proteins. It can either be used to ensure that the GST moiety of a fusion protein is accessible to the anti-GST antibody or to determine the concentration of a GST-tagged protein.

Its principle is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown below, GST labeled with XL665 is detected by anti-GST Cryptate (GST-K) conjugate.



The GST-tagged fusion protein to be ascertained competes with the GST-XL665 conjugate for the binding to the GST-K conjugate. The HTRF® signal (i.e. energy transfer) is inversely proportional to the concentration of GST-tagged fusion protein.

2. Background

The development of fusion protein technology has boosted the use of toolbox reagents for the purification and the detection of recombinant proteins. This technique consists of the addition of a specific sequence (i.e. tag) to the protein to be expressed. These tags can be inserted at different places in the sequence and are often added to N or C-terminal ends to guarantee the production of a biologically active recombinant protein. The protein can then be detected through the tag using toolbox reagents (e.g. antibodies raised against this tag or proteins having an affinity for it). Glutathione S-transferase – an enzyme involved in animal cell detoxication – is one of the tags most widely used in molecular biology.

Both eukaryotic and bacterial expression vectors for the production of GST tagged proteins are commercially available. Expressed fusion proteins can be purified by immobilized glutathione affinity chromatography, the GST moiety being removable if necessary via a specific enzymatic cleavage site introduced in the construct (e.g. thrombin). When tagged with GST, fusion proteins can be easily detected by anti-GST specific antibodies.

3. Protocol

3.1. Supplied reagents and reconstitution

Supplied reagents	Reagent reconstitution (stock solutions)		Working solutions
Anti-GST-Cryptate	1 vial Lyophilized*	⇨	Add 2.5 mL of reconstitution buffer to each vial. Mix gently.
GST-XL665	1 vial Lyophilized*		
GST standard. Concentrated free GST. See label indications for GST concentration after reconstitution.	1 vial Lyophilized*	⇨	See indications on label for reconstitution volume (see Standard curve preparation for further dilution). Mix gently
Diluent 50 mM Phosphate buffer, pH 7.0, 0.2% BSA, preservatives, NaN_3	1 vial of 20 mL		
Reconstitution buffer 50 mM Phosphate buffer, pH 7.0, 0.8M KF	1 vial See volume on the label		

Cisbiop microplates** - Not provided	Part#
96-well low volume plate	66PL96001
384-well low volume plate	66PL384025

* All reagents were lyophilized in 50 mM phosphate buffer, pH 7, containing BSA protease free and stabilizers.
**For HTRF microplate recommendations, please visit http://www.cisbio.com/microplate-recommendation

Allow the reagents to warm up at room temperature for at least 30 minutes and reconstitute all vials as indicated above.

Precaution : HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the XL665 and Cryptate-conjugates will impair the assay's quality.

3.2. Reagent stability

All reagents should be stored at 2-8°C until reconstituted. Under proper storage conditions, they are stable until the expiry date indicated on the labels.

Once reconstituted, the reagents should be kept at 2-8°C for no longer than 2 days. They may also be frozen and stored at –20°C for no longer than 2 months. Avoid repeated freezing and thawing.

3.3- Standard curve preparation

The different standards are made by diluting the main standard with the diluent. The table below indicates how to carry out for the preparation of one Standard curve :

Standard	Preparation	GST concentration in nM
Std 6	Reconstituted reagent (pure)	[GST] *
Std 5	↘ 50 µL Std 6 + 150 µL diluent	[GST] / 4
Std 4	↘ 50 µL Std 5 + 200 µL diluent	[GST] / 20
Std 3	↘ 50 µL Std 4 + 200 µL diluent	[GST] / 100
Std 2	↘ 100 µL Std 3 + 100 µL diluent	[GST] / 200
Std 1	↘ 50 µL Std 2 + 200 µL diluent	[GST] / 1000

* [GST] is indicated on the label of the maximum standard. It corresponds to the concentration of the solution obtained after reconstitution with distilled water.

3.4. Sample preparation

Dilute all samples to be assayed with the diluent. Consecutive dilutions should be made within the 1 to 400 nM range (working solution).

3.5. Assay protocol for 384-well low volume plate

Dispense the reagents in the following order :

10 µL standard or sample
5 µL Anti-GST-Cryptate
5 µL GST-XL665

For negative control, replace the standard by 10 µL of diluent and the GST-XL665 by 5 µL of reconstitution buffer.

For positive control, replace the first reagent by 10 µL of diluent.

⇨ Cover the plate with a plate sealer and leave to incubate at room temperature for 2 hours.

⇨ Read on a compatible HTRF® reader (more information about compatible reader at htrf-assays.com/readers).

3.6. Data reduction

Results are calculated from the 665nm / 620nm ratio and expressed in Delta F. An example of data reduction is given in the table below (readout on PHERAstar Plus). This data should not be substituted for results obtained in the laboratory. Draw up the Standard curve by plotting delta F % versus GST concentrations. Deduce the concentration of the sample assayed from the curve obtained. An example of the displacement curve is given on the right.

	A (665nm)	B (620nm)	Ratio (1)	Mean Ratio (2)	CV % (3)	Delta F % (4)
Negative control	1839 2007	44229 49005	416 410	413	1.1	
[Standard nM initial						
0 Positive control	32961 33181	50254 49524	6559 6700	6629	1.5	1506
0.4	32361 33413	50348 54492	6427 6132	6280	3.3	1422
2	29425 26373	51990 48599	5660 5427	5543	3.0	1243
4	23558 27033	50598 60854	4656 4442	4549	3.3	1002
20	11349 11106	54351 53747	2088 2066	2077	0.7	403
100	5078 5542	56185 60945	904 909	907	0.4	120
400	3394 3641	61012 63831	556 570	563	1.8	37

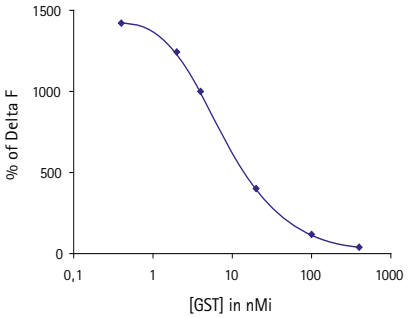
1. $\text{Ratio} = \frac{A_{665nm}}{B_{620nm}} \times 10^4$

2. $\text{Mean Ratio} = \frac{\Sigma \text{ratios}}{2}$

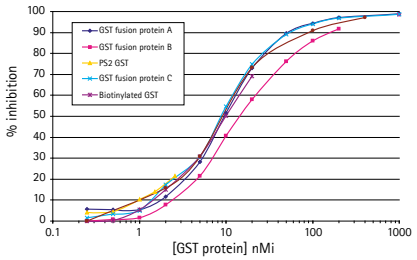
3. $\text{CV} = \frac{\text{Std deviation}}{\text{Mean ratio}} \times 100$

4. $\text{Delta F} = \frac{\text{Calibrator or sample Ratio} - \text{Ratio}_{neg}}{\text{Ratio}_{neg}} \times 100$

(Ratio_{neg} = negative control)



The GST check kit enables the verification of tag accessibility on GST-tagged fusion proteins. The graph alongside shows the dose response curves of different GST fusion proteins.



3.7. Assay characteristics

The table summarizes the characteristics of the assay relative to the detection limit (GST concentration corresponding to the "dose of mean zero - 2SD") and the EC_{50} (GST concentration which allows the displacement of 50% of binding). This data has been obtained using the reference PHERAstar Plus reader (BMG LABTECH).

Detection limit	EC_{50}
0.26 nM	10 nM