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# Product information:

Document reference : 62GSTPEB rev04 (July 2008)

# *GST-tag check kit*

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

Storage temperature : 2-8°C

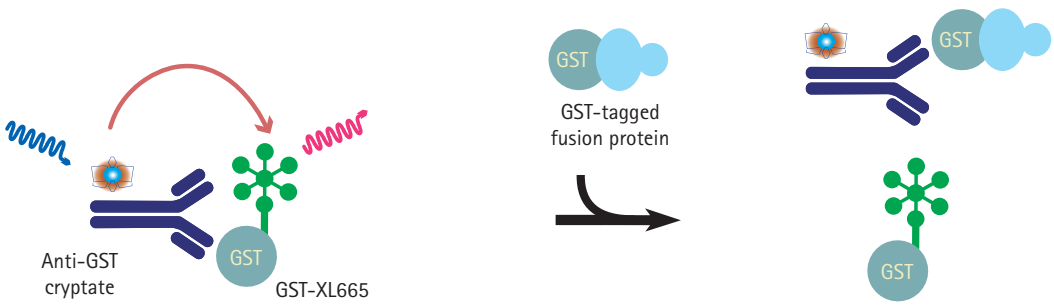
**Packaging details :**

| | 384-well low volume plate (20 µL) |
|---|---|
| 62GSTPEB | 1,000 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 5 mL of reconstitution buffer to each vial. Mix gently. |
| GST-XL665 | 1 vial Lyophilized* | | |

| Supplied reagents (continued) | Reagent reconstitution (stock solutions) | | Working solutions |
|---|---|---|---|
| GST calibrator. Concentrated free GST.<br>See label indications for GST concentration after reconstitution. | 1 vial<br>Lyophilized* | ⇨ | See indications on label for reconstitution volume (see calibration curve preparation for further dilution). Mix gently |
| GST control. Prediluted free GST.<br>See label indications for GST concentration after reconstitution. | 1 vial<br>Lyophilized* | ⇨ | See indications on label for reconstitution volume . Mix gently. |

| | |
|---|---|
| Reconstitution buffer<br>50 mM Phosphate buffer, pH 7.0, 0.8M KF | 1 vial<br>See volume on the label |
| Diluent<br>50 mM Phosphate buffer, pH 7.0, 0.2% BSA, preservatives, $NaN_3$ | 1 vial of 20 mL |

* All reagents were lyophilized in 50 mM phosphate buffer, pH 7, containing BSA protease free and stabilizers.

** The reconstitution and preparation procedures of the Cryptate conjugate working solution (given below) were optimized for a maximum assay sensitivity and to ensure an average counting of 40,000 cps at 620 nm (384-well low volume format), using the reference PHERAstar Plus reader (BMG LABTECH).

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- Calibration curve preparation

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

| Calibrator | Preparation | GST concentration in nM |
|---|---|---|
| Cal 6 | Reconstituted reagent (pure) | [ GST ] * |
| Cal 5 | ⇘ 50 µL Cal 6 + 150 µL diluent | [ GST ] / 4 |
| Cal 4 | ⇘ 50 µL Cal 5 + 200 µL diluent | [ GST ] / 20 |
| Cal 3 | ⇘ 50 µL Cal 4 + 200 µL diluent | [ GST ] / 100 |
| Cal 2 | ⇘ 100 µL Cal 3 + 100 µL diluent | [ GST ] / 200 |
| Cal 1 | ⇘ 50 µL Cal 2 + 200 µL diluent | [ GST ] / 1000 |

* [GST] is indicated on the label of the maximum calibrator. 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 calibrator or control or sample*
- 5 µL Anti-GST-Cryptate
- 5 µL GST-XL665

* For negative control, replace the calibrator 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. Assay flexibility and miniaturization

When used as suggested, the kit will provide sufficient reagents for 1,000 tests using using a 384- well low volume plate in 20 µL final assay volume (HTRF® packaged basis).

To move to other plate formats (96 half-well or 1536-well) and final volumes (100 µL to less than 10 µL), the volume of each assay component is simply proportionally adjusted in order to maintain the reagent concentrations as for the 20 µL final assay volume. For instance, in the case of the 1536-well format in 10 µL final volume, half as much material per well is used, thereby allowing 2,000 tests to be run. The performances of the HTRF® assay remain the same whatever the level of miniaturization.

| Assay components | Volume proportion | Assay format | | |
|---|---|---|---|---|
| | | 1536-well (10 µL) | 384-well low volume (20 µL) | 96 half-well (100 µL) |
| Sample | 2 volumes | 5 µL | 10 µL | 50 µL |
| XL665 conjugate | 1 volume | 2.5 µL | 5 µL | 25 µL |
| Cryptate conjugate | 1 volume | 2.5 µL | 5 µL | 25 µL |
| | | 2,000 tests | 1,000 tests | 200 tests |

Plate references : 96 half-well plate (Costar # 3694 or equivalent), 384-well low volume plate (Greiner # 784076), 1536-well (Greiner # 782086)

## 3.7. 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 calibration 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.

The GST control validates the accuracy of the calibration curve. The concentration deduced from the Delta F obtained should fall into the concentration range indicated on the label of the vial.

| | A (665nm) | B (620nm) | Ratio (1) | Mean Ratio (2) | CV % (3) | Delta F % (4) |
|---|---|---|---|---|---|---|
| Negative control | 1839<br>2007 | 44229<br>49005 | 416<br>410 | 413 | 1.1 | |
| **[calibrator] nM initial** | | | | | | |
| 0<br>Positive control | 32961<br>33181 | 50254<br>49524 | 6559<br>6700 | 6629 | 1.5 | 1506 |
| 0.4 | 32361<br>33413 | 50348<br>54492 | 6427<br>6132 | 6280 | 3.3 | 1422 |
| 2 | 29425<br>26373 | 51990<br>48599 | 5660<br>5427 | 5543 | 3.0 | 1243 |
| 4 | 23558<br>27033 | 50598<br>60854 | 4656<br>4442 | 4549 | 3.3 | 1002 |
| 20 | 11349<br>11106 | 54351<br>53747 | 2088<br>2066 | 2077 | 0.7 | 403 |
| 100 | 5078<br>5542 | 56185<br>60945 | 904<br>909 | 907 | 0.4 | 120 |
| 400 | 3394<br>3641 | 61012<br>63831 | 556<br>570 | 563 | 1.8 | 37 |
| GST control | 21740<br>19436 | 57656<br>53523 | 3771<br>3631 | 3701 | 2.7 | 797 |

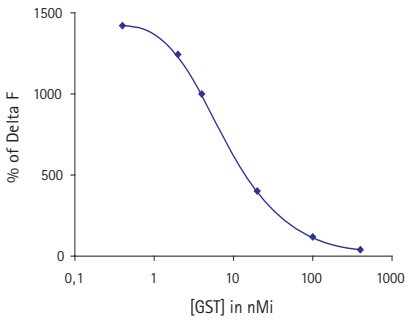
1. $\text{Ratio} = \frac{A_{665nm}}{B_{620nm}} \times 10^4$

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

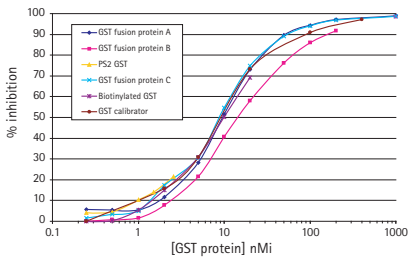
3. $\text{CV} = \frac{\text{Std deviation}}{\text{Mean ratio}} \times 100$

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

($\text{Ratio}_{neg}$ = 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.




## 3.8. 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 |

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