PROTOCOL

GST Tag Check&Go!

Applicable to: 4005-0030 Release 1 © EXPEDEON. 01/05/2019

INTRODUCTION

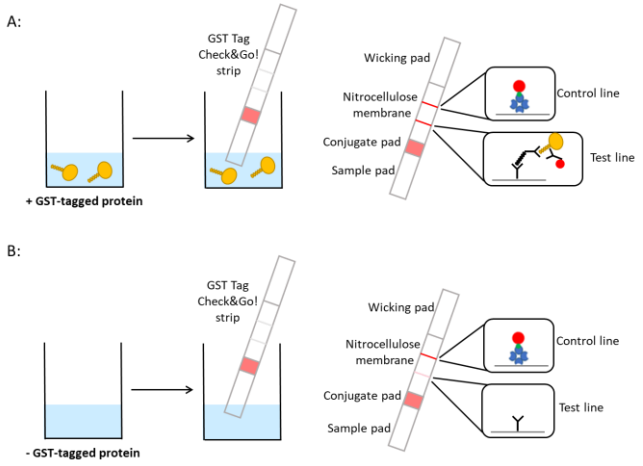
Protein tags are used in the production of recombinant proteins to facilitate downstream purification. GST Tag is a commonly used purification tag, consisting of a 220 amino acid sequence which can be attached to the amino- or carboxyl- terminus of recombinant proteins. Proteins labelled with a GST Tag can subsequently be purified using glutathione affinity resins, followed by an optional cleavage step.

GST Tag Check&Go! (4005-0030) is formulated as a qualitative sandwich lateral flow assay using the Capt*Sure*™ technology; the conjugate pad contains a mixture of anti-GST antibody conjugated to either 40nm gold nanoparticles or Capt*Sure*™ peptide whilst the "Test line" (T line) on the nitrocellulose contains an antibody which binds the Capt*Sure*™ peptide. As such, a strong T line will appear in the presence of GST-tagged proteins (Figure 1- A) whereas in the absence of GST-tagged protein the T line will not be visible or will appear as a faint line (Figure 1-B).

The conjugate pad of the GST Tag Check&Go! strips also contains biotin-gold, which binds streptavidin on the "Control Line" (C line) of the nitrocellulose strips. Hence, the C line is assay-independent and should always appear as a strong red line; if it is not visible, the test is not valid and should be repeated.

Each kit contains a vial of freeze-dried GST-tagged protein, which can be used as a positive control for the lateral flow assay.

FIGURE 1.



SAMPLE CONSIDERATIONS

The strips are compatible with cell culture media and lysate, as well as most common components used in purification processes (Appendix). It is advisable to run the samples in duplicate and to include a sample without GST-tagged protein as negative control, to allow users to determine the background signal intensity on the T line. Finally, it is also recommended to test the strips with the positive control provided in the kit, to confirm a positive signal. The C line should always have the same signal intensity.

KIT CONTENTS

- 2 cryovials of 10X Running Buffer (1.5ml)
- 30 GST Tag Check&Go! strips
- 1 glass vial of GST positive control

Not supplied: 96-well low binding plate, MilliQ water

STORAGE AND SHIPPING

The kit is shipped at ambient temperature. Upon receipt, store the strips at +4°C and the 10X running buffer and GST positive control at -20°C.

INSTRUCTIONS

1. Bring all the kit components to room temperature.
2. Dilute the 10X Running Buffer to a 1X working solution in MilliQ water.
3. Only 80ul of diluted sample is required for each strip. Dilute the lysate/sample containing the GST-tagged protein down to 1µg/ml-100ng/ml in 1X Running Buffer; the appropriate range is protein dependent and should be determined empirically. If the protein concentration in the sample is unknown, perform serial 1:10 dilutions in 1X Running Buffer and test more than one dilution.
4. Reconstitute the freeze-dried positive control in 100µl of 1X Running Buffer to obtain a 100ug/ml stock.
5. Dilute the positive control stock to 100ng/ml by performing a 1/100 dilution, followed by a 1/10 dilution in 1X Running Buffer.
6. Load 80µl of diluted protein into a 96 well clear plate (low protein binding) and dip the end of the strip with the sample pad (see Figure 1) into the liquid.
7. Wait 15-20 minutes for the T and C lines to develop (do not allow the strips to dry out before checking the result).
8. Check the strips; for a positive result the T line intensity should be equivalent or stronger than the C line (see positive control). If the T line intensity is similar to the negative control, or no T line is present, there is insufficient GST-tagged protein in the sample to give a definitive result

ASSAY CONSIDERATIONS

1. The strips are single-use.

2. Always store the unused strips in the closed desiccant pot to prevent moisture from compromising their functionality.

3. Make sure the flow is consistent and that both the sample pad overlapping the conjugate pad and the conjugate pad overlapping the nitrocellulose membrane are making physical contact. If not, a slight bend of the strip (avoiding touching or damaging the nitrocellulose membrane) is enough to restore the contact and establish a steady and effective flow.

4. For optimal T line intensity, samples should be diluted down to a concentration of 1µg/ml-100ng/ml of GST-tagged protein.

APPENDIX

Assay compatibility and interfering substances:

Additive	Acceptable concentration
1X PBS	Fully compatible
1X TBS	Fully compatible
RIPA buffer	Fully compatible
Tween20	< 2%
CHAPS	< 1%
Triton	< 0.5%
SDS	< 0.01%
Imidazole	< 50mM
Guanidine HCl	< 1mM
Urea	< 125mM
DTT	< 20mM
2-mercaptoethanol	< 50mM
EDTA	< 50mM

TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team at: technical.enquiries@expedeon.com

For further information see our website: www.expedeon.com

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