PROTOCOL

HisTag Check&Go!

Applicable to: 4003-0030

Release 1 © EXPEDEON 24/09/2018

INTRODUCTION

HisTag, a string of six to nine histidine residues, is one of the most popular epitope tags used in the production of recombinant proteins as it simplifies their purification in the downstream processes. The poly-HisTag can be fused to the amino- or carboxyl- terminus of the recombinant protein and can bind to multiple metal ions, such as nickel, cobalt and copper, immobilized on purification resins.

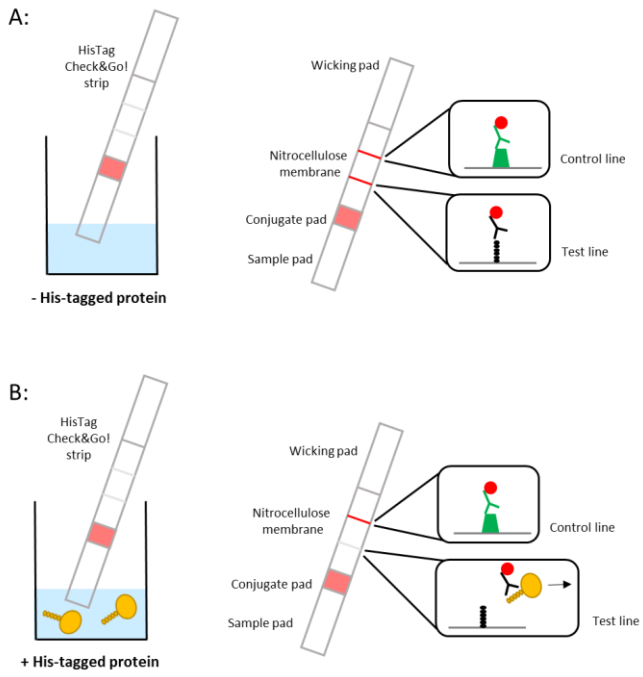
HisTag Check&Go! is a quick competitive immunochromatography test that allows users to verify and monitor successful expression of His-tagged recombinant proteins before undertaking purification, as well as identifying fractions of most interest before performing other more labor intensive techniques such as Western blot or ELISA.

The assay provides a qualitative yes/no answer, although a semi-quantitative measurement of the protein abundance in the sample can be determined using internal references and controls.

A His-tagged protein on the “Test line” (T line) and a ULFA-tagged protein on the “Control line” (C line) are immobilized on the nitrocellulose strips.
40nm InnovaCoat® GOLD anti-HisTag antibody and 40nm InnovaCoat® GOLD anti-ULFA tag conjugates are dried on the conjugate pad and will selectively bind to the T and C line, respectively. 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.

HisTag Check&Go! is formulated as a competitive assay so that when the sample does not contain any His-tagged protein, the T line will appear as a strong red line (Figure 1- A). On the contrary, the T line will not be visible (or appear as a pale red line) if the sample contains the His- tagged protein that competes with the binding of the InnovaCoat® GOLD anti-HisTag antibody conjugate to the antigen immobilized on the T line (Figure 1- B).

Figure 1.



SAMPLE CONSIDERATIONS

The HisTag Check&Go! kit has been optimized with proteins containing accessible consecutive histidine regions at the amino- or carboxyl- terminus exposed in their native/ assay conformation.

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.

A sample without the His-tagged protein should always be added as negative control: the strip will show the highest signal intensity on the T line that you can expect. The C line should always have the same signal intensity. To confirm the presence of expressed His-tagged protein in your sample, a decrease in signal intensity of the T line should be seen, as compared to your negative control sample.

Reference samples of known His-tagged protein concentration can be used for a semi-quantitative analysis.

KIT CONTENTS

- 30 HisTag Check&Go! Strips
- 1 cryovial of 10X Running Buffer

Not supplied: 96-wells low binding plate

STORAGE AND SHIPPING

The kit is shipped at ambient temperature. Upon receipt, store the kit at +4°C.

INSTRUCTIONS

1. Bring all the kit components to room temperature.
2. Dilute the 10X Running Buffer to a 1X working solution in water.
3. Only 80ul of diluted sample are required for each strip. Dilute the lysate/sample containing the His-tagged protein down to 0.01-0.5mg/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 1:2 or 1:3 dilutions in 1X Running Buffer.
4. Load 80ul 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.
5. Wait 10-15 minutes for the T and C lines to develop (do not let the strips to dry out before checking the result).
6. Check the strips; the intensity of the T lines inversely correlates with the amount of His tagged protein present in the sample.

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.

APPENDIX

Assay compatibility and interfering substances:

1X PBS	Fully compatible
1X TBS	Fully compatible
RIPA buffer	Fully compatible
Tween20	< 5%
CHAPS	< 1%
Triton	< 5%
SDS	< 1%
Imidazole	< 125mM
Guanidine HCl	< 100mM
Urea	< 125mM
DTT	< 100mM
2-mercaptoethanol	< 100mM
EDTA	< 100mM

RELATED PRODUCTS

His-GST positive control, Product code 4100-0003

TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team via our website: www.expedeon.com