Related Items Available from GSI

EW-80012	High binding ELISA Strips plates (8 wellsx12 strips)	10/pk
EW-80050	ELISA Plate Coating buffer concentrate (10X)	50 ml
EW-80051	Phosphate Buffered Saline (PBS, pH 7.4) (20X)	100 ml
EW-80062	ELISA Plate Blocking Buffer , (10X) milk-based	100 ml
EW-80070	Antibody and Conjugate Diluents for ELISA (10X),	50 ml
EW-80080	Wash buffer concentrate (20X) for ELISA	100 ml
EW-80091	TMB substrate (1-component) for ELISA	500 ml
EW-80100	Stop solution for TMB substrate (ELISA) (10X),	50 ml
EW-80150	ELISA Kit for the detection of Mouse Antibodies	1 kit
EW-80155	ELISA Kit for the detection of Rat Antibodies	1 kit
EW-80160	ELISA Kit for the detection of Rabbit Antibodies	1 kit
EW-80166	ELISA Kit for the detection of Sheep Antibodies	1 kit
EW-80170	ELISA Kit for the detection of Human Antibodies	1 kit
EW-80180	ELISA Kit for the detection of Hamster Antibodies	1 kit
EW-80190	Western blot Kit for mouse or rabbit, human or goat antibodies using TMB color substrate	1 kit
EW-80200	Enhanced NuGlo Western blot kit for HRP	1 kit
EW-80220	HRP Conjugation kit	1 kit

Instruction Manual No. M-80300

Protein Biotinylation Kit

Cat. No. EW-80300

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Introduction

Protein (antigen and antibody) biotinylation is widely used in ELISA/EIA as a detection molecule. This provides the development of a very sensitive immunoassay by detecting conjugated biotin using Streptavidin-HRP. Since it is possible to couple several molecules of biotin per molecule of antigen/antibody, sensitivity of the assay cab be enhanced several fold over conventional immunoassay. The availability of secondary reagents (Streptavidin-HRP or streptavidin coated ELISA plates) makes it very convenient to quickly develop sensitive immunoassay.

GSI uses long arm biotin (Aminohexanoyl-biotin N-hydroxysuccinimide; mol. Wt 454) to provide extra flexibility between biotin and the protein. The biotinylation requires a free amino group on the protein/peptide. The conjugating protein should be free from amine containing buffers (Tris, etc.) to improve coupling efficiency.

Reagents Provided in the Kit

1. 1 vial of Biotin (10 mg; Cat # 80301

2. Conjugation Buffer, pH, 8.4, 100 ml, Cat # 80302, (10X) dilute 1:10 with water before use.

3. Stabilizing Buffer; 5 ml., Cat # 80303

Reagents Required But not Provided

1. PBS, pH 7.4 for Dialyzing the conjugate (dissolve 0.26 g KH_2PO4, 2.17 g $Na_2HPO4.7H_2O$, and 8.71 g of NaCl in 1L H2O).
2. Dialysis bag (Cut off size >4,000 kDa)
3. DMF for dissolving biotin

Procedure

1. Dialyze the protein to be coupled in 1X conjugation buffer, extensively (1-5 ml protein solution in 500-1 liter overnight at 4oC shodul be sufficient). The protein concentration should be 1-10 mg/ml. If the protein is in pure form or in saline or in H2O, it is possible to adjust the pH by adding 10X conjugation buffer and omitting dialysis.

2. Dissolve biotin in DMF at 10 mg/ml before use. Add dissolved biotin protein solution at a predetermined ratio to the protein solution slowly under continuous mixing. A ratio of 1:10 (biotin:protein) may be used for goat or rabbit antibodies. Mix it at room temp. for 1 h or overnight at 4oC.

Notes: Biotin mol wt is ~450 daltons. Most proteins and IgG (150,000 dalton or 150 kDa) . Therefore, 1 mg of biotin will equal approx 333 mg of IgG on a molar basis. So 0.5-1 mg biotin per mg of IgG should make it in large excess and allow several molecules of biotin available per molecule of IgG.

3. Dialyzed the biotin-conjugate extensively against PBS at 4oC.

4. Add stabilizing buffer to the dialyzed conjugate (add 2 ml for each 500 ul of starting protein volume).
5. The conjugate can be kept at 4oC for up to 6 months. Avoid freezing and thawing.

Recommended Usage of The Conjugate

The biotin-conjugate can be used directly at a dilution of 1K-100K. High background in ELISA is usually due to the use of excessive conjugate, improper conjugate diluents or blocking of coated antigens. It may be necessary to use BSA (0.1%) or other carrier proteins in diluents to keep the background to an acceptable range.

Biotinylation often requires fine adjustments between the ratios of biotin and conjugating protein. This will depend upon the availability of free amino groups on target protein and the level of biotinylation desired. It is recommended that the user try several concn. of biotin and test the conjugate in a given immunoassay. It is therefore recommended that the user perform several mini-reactions and test conjugates.

The effectiveness of biotinylation reaction can be tested by streptavidin or anti-biotin coated ELISA plates. It is also possible to coat the ELISA plates directly with the biotinylated proteins and then detecting it streptavidin-HRP or anti-biotin antibodies.