

Block endogenous biotin

TR0016.2

Introduction

Biotin (also known as vitamin H) occurs in all living cells. Although the presence of biotin in biological samples is irrelevant in many assay systems, it can cause high background when making use of tetrameric biotin-binding proteins (avidin, streptavidin or Thermo Scientific NeutrAvidin™ Protein) to detect or purify biotin-labeled probes. The following protocol may be used in immunohistochemical staining, Western blotting or ELISA methods to eliminate background caused by endogenous biotin by blocking it before adding the biotin-labeled probe.

Two basic steps are involved in this blocking procedure:

1. Bind all endogenous biotin moieties with excess streptavidin (or equivalent biotin-binding protein); wash thoroughly.
2. Block remaining streptavidin biotin-binding sites with free biotin; wash thoroughly.

The second step is necessary because streptavidin, avidin and NeutrAvidin Proteins are tetrameric proteins, having four biotin-binding sites per molecule. Therefore, the first step in which endogenous biotin is blocked by excess streptavidin must be followed by a second step in which excess biotin-binding sites on the bound streptavidin are blocked with free biotin. If this second step were not performed, the blocking streptavidin would undesirably bind the biotin-labeled probe used in the assay. After the final wash, the result is a sample in which all biotin molecules are bound by streptavidin and all biotin-binding sites on streptavidin are bound by biotin.

Materials Required (and suggested Thermo Scientific Products)

- Wash Buffer: Tris buffered saline (TBS, Product No. 28376; 25 mM Tris, 150 mM NaCl, pH 7.2) with or without 1-2% bovine serum albumin (BSA, Product No. 37520) and/or 0.05% Tween®-20 (Product No. 28320)
- Streptavidin or other biotin-binding protein: Streptavidin (Product No. 21122, 21125), NeutrAvidin Protein (Product No. 31000) or Avidin (Product No. 21121, 21128)
- Biotin: D-Biotin (Product No. 29129)

Procedure

1. Block sample as usual with a protein-based blocker (e.g., normal serum or Blocker™ BSA in TBS, Product No. 37520).
2. Cover sample with 0.1 mg/ml (~1.7 μM) streptavidin diluted in Wash Buffer; incubate 15 minutes at room temperature.
3. Wash three times for 10 minutes each with Wash Buffer.
4. Add 0.5 mg/ml (~2 mM) Biotin dissolved in Wash Buffer; incubate for 30-60 minutes at room temperature.
5. Wash three times for 10 minutes each with Wash Buffer.
6. Continue with usual assay procedure by adding biotin-labeled probe (e.g., biotinylated antibody).

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Current versions of product instructions are available at www.thermo.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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