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A Geno Technology, Inc. (USA) brand name

Protein-Free Blocking Buffer

INTRODUCTION

Protein-Free Blocking Buffer does not contain protein; it is a proprietary formation of none-protein agents that eliminates non-specific binding sites in ELISA, blotting, immunohistochemistry and other applications. The absence of protein from the *Protein-Free* Blocking Buffer eliminates problems associated with traditional protein based blockers, such as cross-reactivity and interference from glycosylated proteins. *Protein-Free* Blocking Buffer eliminates any concern associated with regulatory compliance issues where use of animal source components are restricted. Furthermore, *Protein-Free* Blocking Buffer is compatible with antibodies and avidin/biotin based systems and results in high signal to background ratios.
For user's convenience *Protein-Free* Blocking Buffers are supplied in widely used TBS (Tris-buffered saline at pH 7.5) and PBS buffers (phosphate-buffered saline at pH 7.5) as well as in separate formulations containing Tween®-20 for improving blocking efficiencies.

KIT COMPONENTS

Cat. #	Description	Size
786-662	*Protein-Free* Blocking Buffer TBS *(in Tris-buffered saline at pH 7.5)*	500ml
786-663	*Protein-Free* Blocking Buffer TBST *(in Tris-buffered saline at pH 7.5 with 0.05% Tween®-20)*	500ml
786-664	*Protein-Free* Blocking Buffer PBS *(in phosphate-buffered saline at pH 7.5)*	500ml
786-665	*Protein-Free* Blocking Buffer PBST *(in phosphate-buffered saline at pH 7.5 with 0.05% Tween®-20)*	500ml

STORAGE CONDITIONS

Shipped at ambient conditions, upon arrival store at 4°C.

IMPORTANT

- For optimal blocking, do NOT dilute the *Protein-Free* Blocking Buffer.
- The efficacy of blocking agents varies from application to application, so we recommend empirical testing of blocking buffer and optimization of procedure to increase sensitivity and prevent nonspecific signal and cross-reaction between blocking agent and antibody.
- Use of detergent in blocking buffers is not required for all applications, however, addition of 0.05% Tween®-20 often improves blocking. Use only high quality ultra pure grade Tween®-20, we recommend our *Proteomic Grade* Tween®-20 solution (Cat. # DG011, DG012, DG511), which is purified to remove peroxide and carbonyls contaminants that may interfere in some applications. Do not add additional detergent to *Protein-Free* Blocking Buffer in TBST (Cat. # 786-663) or *Protein-Free* Blocking Buffer in PBST (Cat. # 786-665) as these have optimal Tween®-20.
- *Protein-Free* Blocking Buffer may be used as stabilizer of proteins coated on ELISA plates for storage.

PROCEDURE FOR BLOCKING WESTERN BLOTTING MEMBRANES

1. Following protein transfer to the membrane, transfer the membrane to a suitable size tray.
   NOTE: Protein-Free Blocking Buffer is suitable for PVDF and nitrocellulose membranes.

2. Add enough *Protein-Free* Blocking Buffer to completely cover the membrane.

3. Incubate for 60-120 minutes at room temperature with agitation.

4. Discard blocking buffer and continue with downstream Western blotting steps.
   NOTE: For washing steps, use of femtoTBST™ (Cat.# 786-161) or femtoPBST™ (Cat. # 786-162) will minimize the washing out of immune-complexes and aid in the generation of cleaner backgrounds resulting in a higher signal to noise ratio, a common problem associated with classical TBST and PBST buffers used for washing.

PROCEDURE FOR BLOCKING ELISA PLATE

1. Apply sample (antigen or antibody) to the ELISA plates and incubate for 1-2 hours at room temperature.

2. Apply 300µl of *Protein-Free* Blocking Buffer to each well. Immediately empty the well by aspiration or inversion. Repeat this step twice more. Incubation is not required, however plates may be incubated without any detrimental effects.

3. Continue the downstream ELISA steps.
   NOTE: For washing steps, use of femtoTBST™ (Cat.# 786-161) or femtoPBST™ (Cat. # 786-162) will minimize the washing out of immune-complexes and aid in the generation of cleaner backgrounds resulting in a higher signal to noise ratio, a common problem associated with classical TBST and PBST buffers used for washing.

4. For storage of coated plates, invert plates and allow plates to dry completely before sealing in a plastic bag with desiccant.

PROCEDURE FOR BLOCKING TISSUE FOR IMMUNOHISTOCHEMISTRY

1. Incubate tissue in blocking buffer for 30 minutes at room temperature.

2. Remove the blocking buffer from the tissue.

3. Without rinsing the tissue, continue with immunohistochemistry downstream procedures for detection

RELATED PRODUCTS

I. ***Proteomic Grade*** **Tween®-20 Solution** (Cat. # DG011, DG012, DG511): *Contain reduced peroxides and carbonyl compounds. These detergents are offered as 10% aqueous solutions, sealed under inert gas and are suitable for protein applications.*

II. ***femto*****TBST™** (Cat.# 786-161) and ***femto*****PBST™** (Cat. # 786-162): *Enhance sensitivity of your immunoassays by minimizing the "washing out" of immuno agents or immune-complexes, a common problem associated with classical TBST and PBST buffers. The wash buffers aid in the generation of cleaner backgrounds, resulting in a larger ratio of signal to background.*

III. ***Swift™*** **Membrane Stain** (Cat. # 786-677): *A unique, proprietary (patents pending), reversible, ready-to-use membrane stain for proteins on nitrocellulose or PVDF membranes. Swift Membrane Stain™ stains proteins as swift as the routinely used Ponceau-S stain, but is a more sensitive and stronger stain allowing for greater protein detection and simpler image capture.*

For additional blocking agents, Western blotting and ELISA products, visit www.GBiosciences.com.

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