



Promega

Technical Bulletin

SAM²® Biotin Capture Membrane

INSTRUCTIONS FOR USE OF PRODUCTS V2861 AND V7861.



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SAM²® Biotin Capture Membrane

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1. Description

The SAM²® Biotin Capture Membrane^(a) binds biotinylated molecules based on their affinity for streptavidin. The proprietary process by which the SAM²® Membrane is produced results in a high density of streptavidin on the filter, providing rapid, quantitative substrate binding in the nmol/cm² range. In addition, the membrane has been optimized for low nonspecific binding. Figure 1 outlines the procedure for use of the SAM²® Biotin Capture Membrane with biotinylated substrate molecules as used in our SignaTECT® Protein Kinase Assay Systems.

The SAM²® Membrane is available either as a large, prenumbered, partially cut sheet (approximately 10.5 × 15.0cm; Cat.# V2861) or as a smaller, uncut sheet (approximately 7.6 × 10.9cm; Cat.# V7861). The partially cut SAM²® Membrane (Cat.# V2861) allows easy separation into 96 individual squares and is designed for small-scale experiments where high binding capacity is required. The uncut sheet (Cat.# V7861) can be analyzed as a whole membrane or may be cut into the size desired. The uncut membrane allows for sample application using a multichannel pipettor. Both membranes may be analyzed using phosphorimaging, autoradiography or scintillation counting to quantitate results. The membranes have also been used successfully with chemiluminescence detection techniques. The use of fluorescence for detection of captured molecules has recently been demonstrated using IRDye™-labeled antibodies and the Odyssey® Imaging System.

The SAM²® Membrane, used as recommended in this technical bulletin, provides a number of advantages over other commercially available streptavidin products. These advantages include:

- **Versatility:** Analysis of biotinylated substrates can be applied to a wide variety of substrate types without the need to optimize each substrate for binding to a matrix. Available in 96-square (partially cut) and solid-sheet (uncut) formats, the user can perform experiments with a wide array of sample numbers and sizes without changing the analysis technique.
- **Specificity:** The combination of protein denaturant and high-salt washes minimizes nonspecific binding to the Membrane without interfering with the high affinity interaction between streptavidin and biotin.
- **High Signal-to-Noise Ratios:** The stringent washing conditions employed assist in attaining very low background counts.

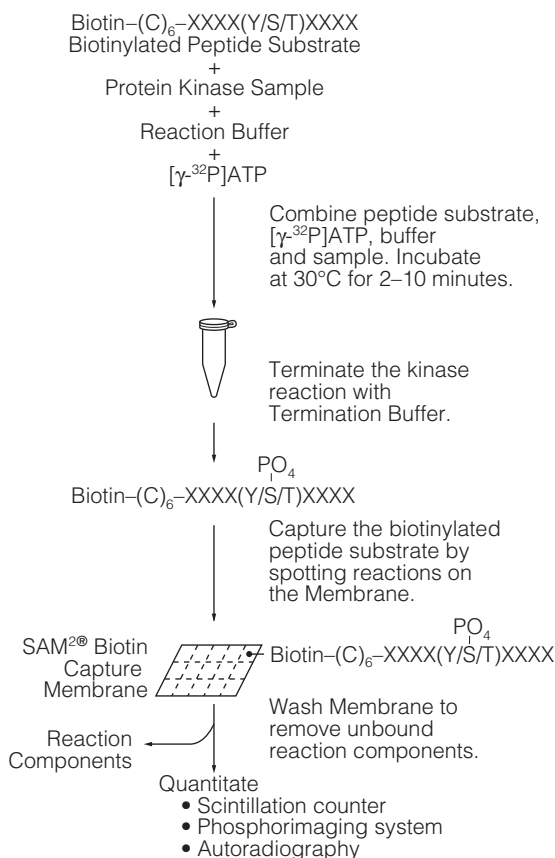


Figure 1. Flow diagram depicting use of the SAM²® Biotin Capture Membrane for analysis of kinase activity as used in our SignaTECT® Protein Kinase Assay Systems.

2. Product Components and Storage Conditions

Product	Size	Cat.#
SAM ² ® Biotin Capture Membrane	96 samples	V2861
SAM ² ® Biotin Capture Membrane (uncut) For Laboratory Use.	7.6 × 10.9cm	V7861

Storage Conditions: Store the SAM²® Biotin Capture Membrane in the resealable bag at -20°C.

3. SAM²® Biotin Capture Membrane Background and Characteristics

3.A. Background

It is frequently desirable in molecular biology and enzymatic analysis to separate a specific substrate from other compounds in a reaction mix. This separation is usually accomplished using a solid matrix that selectively binds the substrate. The matrices, which are often based on ionic or metal ion interactions, are only grossly selective and thus are prone to undesirable variations in performance when different substrates, enzymes or washing conditions are used (1,2). Potential problems include variations in: 1) background and signal intensities depending upon the degree of washing; 2) binding affinity of the substrate based on composition; 3) substrate specificity due to alteration of the substrate to achieve efficient matrix binding. In addition, variations in performance with standard matrices can occur due to the detection of signal from miscellaneous substrates present in complex samples such as crude cell or tissue extracts, which can bind nonspecifically to the matrix (2,3).

Some assays have circumvented these problems by using the high-affinity streptavidin:biotin interaction ($K_d = 10^{-15}M$) to separate the substrate from other reactants (4). This has been accomplished using biotinylated substrates and streptavidin-coated plates or beads. Unfortunately, the limited capacity of streptavidin-coated plates and streptavidin-coated beads places restrictions on the parameters of the assay, thereby limiting the utility of these formats. For example, many enzymes, particularly protein tyrosine kinases, have high K_m values for peptide substrates, frequently above 25 μM and as high as 1mM (5). To work at maximal sensitivity (near V_{max}) the peptide substrate concentrations must be at least 3 times greater than the K_m . The binding capacity of commercially available streptavidin-coated plates and beads is generally at least one order of magnitude below this desired capacity. Even if the enzyme activity were sufficiently high to allow suboptimal substrate concentrations to be used at the 96-sample level, further restrictions would be encountered when using miniaturization to 384 samples or higher density arrays.

3.B. Membrane Characteristics

The SAM²® Membrane overcomes the problems described above by providing the binding capacity to work at optimal conditions at the 96-sample level while retaining sufficient signal-to-noise ratios to allow miniaturization to higher sample density arrays. The binding of biotin to streptavidin is rapid and strong; binding of the biotinylated molecules to the SAM²® Membrane occurs within 30 seconds of sample application. Once formed, this association is unaffected by extremes in pH, temperature, organic solvents, ionic and nonionic detergents and denaturing agents (Table 1; 4).

The 96-square-sheet format (Cat.# V2861) is prenumbered and partially cut so that individual squares can be easily identified, separated and placed into scintillation vials or left intact and quantitated by phosphorimaging or by conventional autoradiography. The uncut sheet (Cat.# V7861) can be utilized for multiple samples in the solid-sheet format, or it can be custom cut to accommodate various sample size and sample number specifications.

Table 1. Stability Data for the SAM²® Biotin Capture Membrane*.

Factor	Range Compatible with the SAM²® Membrane
Organic solvents	95% ethanol
Detergents	1% SDS, 1% CHAPS, 1% Triton® X-100, 1% Tween® 20, 1% Tween® 40
Denaturing agents	5M guanidine hydrochloride, 2M urea
pH	2.0-10.0
Ionic strength	0-5M NaCl
Binding of streptavidin:biotin	$K_d = 10^{-15}M$
Binding time	<30 seconds
Background counts	0.02-0.1%

*See reference 6.

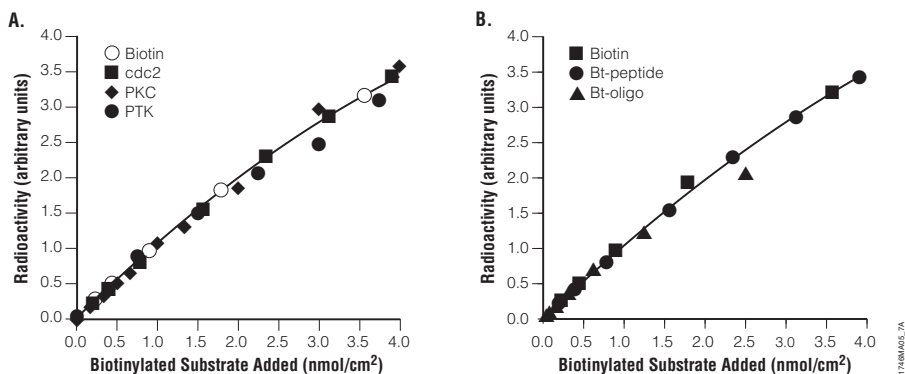



Figure 2. Binding of biotin and various biotinylated substrates to the SAM²® Biotin Capture Membrane. **Panel A.** Binding of several different biotinylated peptide substrates and biotin alone to the SAM²® Membrane was compared. The indicated amounts (X axis) of radioactive biotin and biotinylated peptide substrates for cdc2, PKC and PTK in 2.5M guanidine-HCl were spotted onto the SAM²® Membrane, washed 4X in 2M NaCl, 4X in 2M NaCl plus 1% H₃PO₄ and 2X in water, dried and the results quantitated by scintillation counting. **Panel B.** Radioactive biotin and biotinylated peptide (Bt-peptide) were spotted onto the Membrane and washed as stated above. Radioactive, biotinylated oligonucleotide (Bt-oligo) in water was spotted onto the SAM²® Membrane, washed 4X in 1% SDS, 2X in water, 4X in 2M NaCl and 2X in water, dried and the results quantitated by scintillation counting.

Studies performed in our laboratories have tested the SAM²® Membrane with many different biotinylated peptides and oligonucleotides. We have shown that the binding affinity of the membrane for these substrates is similar to the affinity of the membrane for biotin alone (1-3). In addition, binding of biotinylated molecules to the SAM²® Membrane occurs independently of amino acid or nucleotide sequence (Figure 2). This property allows the comparison of multiple peptide substrates, which is especially important for those peptide substrates that do not bind well to standard matrices (7-9).

4. Procedure for Use of SAM²[®] Biotin Capture Membrane in Kinase Assays

The following procedure is recommended for use of the SAM²[®] Membrane in kinase assays with biotinylated peptide substrates. Please note that you will need to optimize the buffers and washing protocol for use of the membrane with other types of molecules.

1. Wearing gloves, cut (using scissors or a razor blade) the required number of squares from the partially cut SAM²[®] Membrane. Alternatively, the squares may remain connected as a sheet to minimize handling. When working with the uncut membrane (Cat.# V7861), either cut into individual pieces or handle as a whole sheet. Return any unused membrane to the resealable bag at 2–8°C or –15 to –25°C.
2. After completion of the protein kinase reactions, terminate with 0.5 volume of 7.5M guanidine hydrochloride solution in water (final concentration of 2.5M guanidine hydrochloride).
3. When using the partially cut membrane (Cat.# V2861), apply 0.1–25µl of the terminated kinase reaction (≤ 2 nmol of peptide) to an individual membrane square or apply a maximum of 15µl per square if the squares are still connected. For the uncut SAM²[®] Membrane (Cat.# V7861), apply a substrate concentration of ≤ 1.3 nmol/cm² (see Note below). If applying samples with a multichannel pipettor, the maximum volume applied should be ≤ 5 µl. Allow the samples to adsorb to the membrane; there is no need to dry the membrane completely before washing.

 Do not exceed 30µl per square.

Note: It is possible to bind more than 1.3nmol/cm² and retain a linear binding response. The linear binding response above 1.3nmol/cm² will depend upon the assay being performed and must be determined by the user (Figure 2).

4. Place the SAM²[®] Membrane squares or the intact sheet containing samples into a washing container. Wash, using a minimum of 100ml of each solution per sheet, changing solutions after each wash. Using an orbital platform shaker set on low speed or by manual shaking, follow this washing procedure:

Wash 1 time for 30 seconds with 2M NaCl.



Wash 3 times for 2 minutes each with 2M NaCl.



Wash 4 times for 2 minutes each with 2M NaCl in 1% H₃PO₄.



Wash 2 times for 30 seconds each with deionized water.

Total wash time <20 minutes.

Notes:

If using radioisotopes, dispose of the radioactive wash solution in accordance with the regulations of your institution.

More or less washing may be appropriate to achieve acceptably low background counts; this should be determined empirically.

For rapid drying, a final 15-second, 95% ethanol wash can be used. Longer washes with ethanol may cause the ink to run slightly.

5. Dry the SAM²[®] Membrane squares on a piece of aluminum foil under a heat lamp for 5-10 minutes or air-dry at room temperature 30-60 minutes. (If the SAM²[®] Membrane has been washed with ethanol, shorten the drying time to 2-5 minutes under a heat lamp or 10-15 minutes at room temperature.)
6. **Analysis by Scintillation Counting:** If you are using radioisotopes and the SAM²[®] Membrane (Cat.# V2861) is still intact, separate the squares using forceps, scissors or a razor blade and place into individual scintillation vials. Add scintillation fluid to the vials and count. The uncut SAM²[®] Membrane (Cat.# V7861) may be cut into sample pieces and each piece analyzed in individual vials after addition of scintillation cocktail.

Analysis by Phosphorimaging: Alternatively, the SAM²[®] Membrane may remain intact, and the intact membrane may be analyzed using a phosphorimaging system.

5. Related Products

Product	Size	Cat.#
SAM ² [®] 96 Biotin Capture Plate	96-well plate	V7541
	5 × 96-well plates	V7542
SignaTECT [®] Protein Tyrosine Kinase Assay System	96 reactions	V6480
SignaTECT [®] Protein Kinase C (PKC) Assay System	96 reactions	V7470
SignaTECT [®] cAMP-Dependent Protein Kinase (PKA) Assay System	96 reactions	V7480
SignaTECT [®] DNA-Dependent Protein Kinase Assay System	96 reactions	V7870
SignaTECT [®] Calcium/Calmodulin-Dependent Protein Kinase (CaM KII) Assay System	96 reactions	V8161
SignaTECT [®] cdc2 Protein Kinase Assay System	96 reactions	V6430
Kinase-Glo [®] Luminescent Kinase Assay	10ml	V6711
	10 × 10ml	V6712
	100ml	V6713
	10 × 100ml	V6714

5. Related Products (continued)

Product	Size	Cat.#
Kinase-Glo® Plus Luminescent Kinase Assay	10ml	V3771
	10 × 10ml	V3772
	100ml	V3773
	10 × 100ml	V3774
PepTag® Non-Radioactive PKC Assay	120 reactions	V5330
PepTag® Non-Radioactive cAMP-Dependent Protein Kinase Assay	120 reactions	V5340

6. References

1. Casnellie, J.E. (1991) Assay of protein kinases using peptides with basic residues for phosphocellulose binding. *Methods Enzymol.* **200**, 115-20.
2. Toomik, R., Ekman P. and Engstrom L. (1992) A potential pitfall in protein kinase assay: Phosphocellulose paper as an unreliable adsorbent of produced phosphopeptides. *Anal. Biochem.* **204**, 311-4.
3. Cisek, L.J. and Corden, J.L. (1991) Purification of protein kinases that phosphorylate the repetitive carboxyl-terminal domain of eukaryotic RNA polymerase II. *Methods Enzymol.* **200**, 301-25.
4. Savage, D. *et al.* (1992) *Avidin-Biotin Chemistry: A Handbook*, Pierce Chemical Co., Rockford, IL.
5. Kemp, B.E. and Pearson, R.B. (1991) Design and use of peptide substrates for protein kinases. *Methods Enzymol.* **200**, 121-34.
6. Ross, S.E., Carson, S.D. and Fink, L.M. (1986) Effects of detergent on avidin-biotin interaction. *BioTechniques* **4**, 350-4.
7. Goueli, B.S. *et al.* (1995) A novel and simple method to assay the activity of individual protein kinases in a crude tissue extract. *Anal. Biochem.* **225**, 10-2.
8. Goueli, S.A., Schaefer, E. and Tereba, A. (1996) SAM²® Biotin Capture Membrane and SignaTECT® Protein Kinase Assay Systems. *Promega Notes* **58**, 22-9.
9. Schaefer, E., Hsaio, K and Guimond, S. (1996) Detection and quantitation of protein tyrosine kinases. *Promega Notes* **59**, 2-9.

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