Opti-4CN™ Substrate Kit
170-8235
Opti-4CN Detection Kit, Goat-anti-Rabbit
170-8236
Opti-4CN Detection Kit, Goat-anti-Mouse
170-8237
Amplified Opti-4CN Substrate Kit
170-8238
Amplified Opti-4CN Detection Kit, Goat-anti-Rabbit
170-8239
Amplified Opti-4CN Detection Kit, Goat-anti-Mouse
170-8240

Instruction Manual

For Technical Service
Call Your Local Bio-Rad Office or
in the U.S. Call **1-800-4BIORAD**
(1-800-424-6723)

Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547
4100130 Rev C

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Section 1
Preparation

1.1 Introduction

Opti-4CN is an improved and more sensitive version of the colorimetric horseradish peroxidase (HRP) substrate, 4-chloro-1-naphthol (4CN). Opti-4CN may be used simply as a replacement for 4CN resulting in a 4–8 fold increase in detection sensitivity. When used in conjunction with the signal amplification reagents in the Amplified Opti-4CN substrate and detection kits, sensitivity may be improved another 4–8 fold, resulting in an overall improvement of 16–64 fold. The kits provide reagents for amplification and/or detection on 2,500 cm^2 of membrane.

1.2 Method Overview

The first step in western blotting is the transfer of antigen onto a solid support membrane by one of several methods. The transfer can be done electrophoretically, following separation of the antigen in a polyacrylamide or agarose gel, passively by directly spotting the antigen onto a membrane, or by vacuum filtration using a microfiltration apparatus. Following antigen binding, the remaining protein binding sites on the membrane surface are blocked to minimize non-specific interactions.

The membrane with bound antigen is then incubated with a primary antibody specific to the antigen of interest. The blot is washed to remove unbound antibody, incubated with a secondary antibody linked to HRP, and then washed again to remove unbound secondary antibody. If there is no amplification involved (*i.e.*, catalog numbers 170-8235 through 170-8237), then the blot is incubated in the Opti-4CN substrate for up to 30 minutes, or until the desired sensitivity is attained. If the signal is to be amplified, the blot is incubated in the Bio-Rad amplification reagent (BAR), washed, incubated in streptavidin-HRP, and washed again before being incubated in the Opti-4CN substrate.

1.3 Kit Components

170-8235	Opti-4CN Substrate Kit Opti-4CN substrate, 12.5 ml Opti-4CN diluent concentrate, 10x, 62.5 ml
170-8236	Opti-4CN Detection Kit, Goat-anti-Rabbit Opti-4CN substrate, 12.5 ml Opti-4CN diluent concentrate, 10x, 62.5 ml Goat-anti-Rabbit-HRP conjugated secondary antibody, 0.5 ml
170-8237	Opti-4CN Detection Kit, Goat-anti-Mouse Opti-4CN substrate, 12.5 ml Opti-4CN diluent concentrate, 10x, 62.5 ml Goat-anti-Mouse-HRP conjugated secondary antibody, 0.5 ml
170-8238	Amplified Opti-4CN Substrate Kit Bio-Rad Amplification Reagent, 53 ml Streptavidin-HRP, 0.5 ml Blocker, 20 g PBS, powder to make 1 liter of 10x PBS 2x Amplification diluent, 106 ml Opti-4CN substrate, 12.5 ml Opti-4CN diluent concentrate, 10x, 62.5 ml
170-8239	Amplified Opti-4CN Detection Kit, Goat-anti-Rabbit All components of 170-8238 Goat-anti-Rabbit-HRP conjugated secondary antibody, 0.5 ml
170-8240	Amplified Opti-4CN Detection Kit, Goat-anti-Mouse All components of 170-8238 Goat-anti-Mouse-HRP conjugated secondary antibody, 0.5 ml

Additional required items not provided in these kits

All kits	Nitrocelluose or PVDF membrane Primary antibody Tween-20, 10 ml, sufficient for 2,500 cm^2 Bovine serum albumin, 7.5 g, sufficient for 2,500 cm^2
170-8235	Blocker Buffer HRP-linked secondary antibody
170-8236/7	Blocker Buffer
170-8238	Dimethyl sulfoxide (DMSO), 500 ml, sufficient for 2,500 cm^2 HRP-linked secondary antibody
170-8239/40	Dimethyl sulfoxide (DMSO), 500 ml, sufficient for 2,500 cm^2

1.4 Product Storage and Stability

The kit is shipped at 4 °C. Store the unopened kit at 4°C. Powdered blocker, powdered PBS and PBS solutions may be stored at room temperature; all other components are stored at 4 °C. After being put into solution, the blocker should be stored at 4 °C. All kit components are guaranteed for 1 year from the time of receipt.

Antibodies are provided in 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.2, with 1% (w/v) bovine serum albumin and 0.01% thimerosal (sodium ethylmercurithiosalicylate) as a preservative. Avoid freeze-thaw cycles of antibody conjugates which will cause breakdown of product. For best results, aliquot conjugates in appropriate amounts and store at -20 °C.

1.5 Safety Instructions

Please read the entire instruction manual before beginning the protocol.

1. Wear gloves and protective clothing, such as laboratory coats and goggles when preparing and working with the solutions in the protocol. DMSO is an irritant; it is a colorless liquid which is easily absorbed through the skin and mucous membranes. Avoid skin contact with DMSO and inhalation of DMSO mist. Wash exposed skin thoroughly with soap and water.

Note: See Material Data Safety Sheet (MSDS) on DMSO for additional information.

2. Work in well-ventilated areas. Avoid inhalation of vapors when working with solutions containing DMSO.
3. Do not mouth pipet any solution.

Section 2
Protocol

2.1 Experimental Strategy and General Considerations

Temperature. All steps are performed at room temperature (22–25 °C).

Making Solutions. Use only deionized, distilled water to prepare solutions. 1x PBST solutions should be sterile filtered. Do not use azide as a preservative in any solution.

Membrane Selection. This kit has been optimized for detection on pure nitrocellulose membranes and performs equally well with supported nitrocellulose membranes. However, due to the way in which they are manufactured, the surface of some supported nitrocellulose membranes take on a 'wavy' appearance that can result in less pleasing images than the pure nitrocellulose membranes which remain flat throughout the process. The kit may also be used to detect proteins bound to PVDF.

Primary Antibody. Generally when serum or tissue culture supernatants are the source of primary antibody, a 1:100–1:1,000 dilution of the primary antibody in buffer is used for detection of antigens on the membrane surface. For chromatographically purified monospecific antibodies, a 1:500–1:10,000 dilution is used for antigen detection. A 1:1,000–1:100,000 dilution is used when

ascites fluid is the source of antibody. Optimal dilution factors must be determined experimentally. The optimal antibody concentration is usually considered the greatest dilution of antibody reagent still resulting in a strong positive signal without membrane background or non-specific reactions.

Secondary Antibody Conjugates. The protocols in this manual were worked out using Bio-Rad Blotting Grade secondary antibody conjugates diluted as described below. Using an antibody conjugate at a higher concentration may result in an overall increase in background without any improvement in detection sensitivity. Secondary antibody conjugates from other sources may be used, but the optimal dilution may be different from that of Bio-Rad antibody conjugates.

Washes and Incubations. Continuous gentle agitation should be used during all incubations and washes. For best results, a rocking platform should be employed to maintain a uniform exposure of the membrane surface to the solution. Use the smallest possible container to hold the membrane and solutions. When a range of washes is specified, as in 'Wash 2–4x', best results are obtained by doing the maximum number of washes. Acceptable results, though potentially with more background, may be obtained with the minimum number of washes. Blocking and washing steps should be done with 0.25 ml per cm^2 of membrane, *e.g.*, for a mini-blot of 60 cm^2, use 15 ml of solution

for those steps. Antibody incubations should be at 0.1 ml per cm^2, *e.g.*, 6 ml for a 60 cm^2 miniblot. Use dilute BAR and streptavidin-HRP solutions at 85 µl per cm^2 of membrane, *e.g.*, 5 ml for a 60 cm^2 mini-blot. The prepared Opti-4CN reagent is used at 0.25 ml per cm^2 of membrane.

Detergents. Tween-20 is essential in washing to eliminate overall background and non-specific hydrophobic interactions. At 0.1%, Tween-20 will not disrupt binding of primary antibodies to antigens or antigens to the membrane, but will optimize detection sensitivity by eliminating non-specific interactions. Alternative detergents should not be substituted.

2.2 Reagent Preparation

The following reagents should be made upon first receiving the kit.

10x PBST (Phosphate buffered saline/1% Tween-20). Pour the contents of the pouch into 950 ml ddH_2O and stir until dissolved. Add 10 ml Tween-20. Bring final volume to 1 liter with ddH_2O. Store at room temperature.

1x PBST (Phosphate buffered saline/0.1% Tween-20). Combine 100 ml 10x PBST and 900 ml ddH_2O. Sterile filter before use. Store at room temperature.

20% DMSO/PBST Wash. Combine 100 ml DMSO and 400 ml 1x PBST. This is sufficient for 500 cm^2 of membrane.

3% Blocker. While vigorously stirring 665 ml of PBST, very slowly add 20 g powdered blocker. Add the powder a little bit at a time over a period of 30–45 minutes. Continue stirring another 30–60 minutes after all the powder has been added. Slowly warming the solution to 55 °C will help put the blocker into solution, but do not overheat. This can be accomplished by placing the flask with the blocker solution inside a beaker on top of a magnetic stirrer/heater. Pour enough room-temperature water into the beaker to surround the blocker solution as it stirs in the flask. Be careful not to cause the flask with the blocker to float or tip. Slowly heat the surounding water to 55–60 °C. Do not add azide. Store at 4 °C. Warm to room temperature before use.

Make these solutions on the day of the experiment.

Antibody dilution buffer (1% BSA in PBST). Dissolve 0.75 g of BSA in 75 ml rapidly stirring PBST (sufficient for 4 miniblots). Used for dilution of primary and secondary antibodies and for dilution of streptavidin-HRP.

Bio-Rad Amplification Reagent. Prepare 85 µl per cm^2 of membrane. Combine 2 parts 2x Amplification diluent, 1 part 4x BAR and 1 part ddH_2O.

Streptavidin-HRP. Prepare 85 µl per cm^2 of membrane. Dilute 1:1,000 with antibody dilution buffer.

2.3 Quick Guide

Blotting and Antibody Incubation

1. Transfer protein to nitrocellulose membrane by electroblotting, dot blotting or microfiltration. Allow membrane to air dry.
2. Wet membrane in PBST and then wash 2x for 5 minutes each time in PBST.
3. Block membrane in 3% blocker for 1 hour.
4. Wash 2x with PBST for 3–5 minutes.
5. Incubate in appropriately diluted primary antibody for one hour.
6. Wash 2x with PBST 5 minutes each time.
7. Incubate in 1:3,000–1:10,000 dilution of GAx-HRP* secondary antibody for 1 hour.
8. Wash 2x with PBST 5 minutes each time.

Amplification (170-8238/39/40). All other kits, skip to Detection.

9. Incubate membrane in diluted BAR for 10 minutes.
10. Wash 2–4x in 20% DMSO/PBST for 5 minutes each time.
11. Wash 1–2x in PBST for 5 minutes each time.
12. Incubate membrane in diluted streptavidin-HRP for 30 minutes.
13. Wash 2x in PBST for 5 minutes each time.

Colorimetric Detection

14. Mix one part Opti-4CN diluent concentrate with nine parts ddH_2O. Prepare 0.25 ml per cm^2 of membrane.
15. Add 0.2 ml of Opti-4CN substrate per 10 mls of diluent (*e.g.*, combine 1 ml Opti-4CN diluent concentrate with 9 ml ddH_2O and 0.2 ml Opti-4CN substrate). Mix well and pour onto membrane.
16. Incubate membrane with gentle agitation in the substrate for up to 30 minutes or until the desired level of sensitivity is attained.
17. Wash the membrane in ddH_2O for 15 minutes.
18. Document or store membrane.

* GAx-HRP is Bio-Rad's Goat Anti-Rabbit IgG-HRP (Catalog number 170-6515) or Goat Anti-Mouse IgG-HRP (170-6516) or Goat Anti-Human IgG-HRP (172-1050).

2.4 Detailed Protocol

Note: Before beginning, read through the entire procedure.

1. Antigen application. Apply antigen to the membrane surface using one of the three basic methods described below. A small amount of known antigen or primary antibody dotted on one corner of the membrane prior to blocking will produce a positive reaction if the procedure is successful.

a. Electrophoretic blotting. The antigens of interest are electrophoretically transferred to the membrane from a gel (*i.e.* SDS-PAGE, IEF or native gel) using the Trans-Blot®, Mini Trans-Blot®, Trans-Blot Semi-Dry cell or similar device.
b. Microfiltration blotting. The antigens of interest are transferred by a vacuum device such as the Bio-Dot® or Bio-Dot SF onto the membrane. The membrane should be removed from the apparatus for the blocking and all subsequent steps.
c. Dot blotting. Cut the membrane sheet to the appropriate size. Draw a grid on the membrane with a pencil. Wet the dry membrane by slowly sliding the membrane at a 45° angle into the PBST. (PVDF membranes must first be wet in 100% methanol; consult membrane instructions for complete information). Remove the thoroughly wetted membrane from the PBST and dry it on filter paper for approximately 5 minutes. Apply antigen sample to each grid square using a syringe or pipet.

Note: Regardless of the method chosen for antigen application, the membrane should be allowed to dry completely before proceeding to the next step.

2. Wash. Wet the membrane in PBST and then wash the membrane twice in PBST for 5 minutes each time. These washes help to reduce spotted or blotchy background problems.

3. Blocking step. Immerse the membrane at a 45° angle into the blocking solution. Gently agitate the solution using a rocking platform and incubate for an hour or more. Best results for amplification are attained with a 3% solution of the blocker provided with the kits (170-8238/39/40). For non-amplified applications, 5% blotto is an acceptable alternative.
4. Wash. Decant the blocking solution and add PBST to the membrane. Wash for 5 minutes. Repeat the wash with fresh PBST.
5. Primary antibody incubation. Decant the PBST and prepare 0.1 ml of antibody solution per cm^2 of membrane. Dilute the primary antibody in PBST with 1% (w/v) BSA. Incubate 1 to 2 hours with gentle agitation. The optimum conditions of dilution and incubation must be determined experimentally.
6. Wash. Decant the antibody solution and add PBST to the membrane. Wash for 5 minutes with gentle agitation and pour off the wash solution. Repeat the wash with fresh PBST.
7. Secondary antibody incubation. Decant the PBST and prepare 0.1 ml of the secondary antibody solution per cm^2 of membrane. Dilute the secondary antibody 1:3,000–1:10,000 with PBST containing 1% (w/v) BSA. Incubate for 30 minutes to 2 hours with gentle agitation. As noted previously, this protocol was developed using Bio-Rad's blotting grade secondary antibodies (Goat Anti-Rabbit IgG-HRP [Catalog number 170-6515] or Goat Anti-Mouse IgG-HRP [170-6516] or Goat Anti-Human IgG-HRP [172-1050]). Secondary antibody HRP conjugates from other sources may be used, but the optimal dilution will have to be determined experimentally.
8. Wash. Decant the antibody solution and add PBST to the membrane. Wash for 5 minutes with gentle agitation and pour off the wash solution. Repeat the wash with fresh PBST.

Amplification (170-8238/39/40). All other kits, skip to Detection.

9. Amplification. Prepare 85 µl of 1x BAR solution per cm^2 of membrane. Prepare the solution by combining 2 parts 2x Amplification diluent, 1 part 4x BAR and 1 part ddH_2O. Pour PBST off the membrane and add the 1x BAR solution. Incubate for 10 minutes with gentle agitation.
10. Wash. Pour off the BAR solution and wash the membrane 2–4 times for 5 minutes each time with 20% DMSO/PBST. Follow these washes with 1–2 washes in PBST for 5 minutes each time.
11. Streptavidin-HRP. Prepare 85 µl of a 1:1,000 dilution streptavidin-HRP per cm^2 of membrane. Dilute the streptavidin-HRP in the same 1% BSA/PBST solution used to dilute the antibodies. Pour off the PBST wash and add the diluted streptavidin-HRP solution. Incubate for 30 minutes with gentle agitation.

12. Wash. Pour off the streptavidin-HRP solution and add PBST. Wash the membrane for 5 minutes with gentle agitation. Repeat the wash step with fresh PBST.

Colorimetric Detection

13. Prepare 0.25 ml of Opti-4CN diluent solution per cm^2 of membrane by first mixing 1 part Opti-4CN diluent concentrate with 9 parts ddH_2O. For each 10 ml of diluent, add 0.2 ml of Opti-4CN substrate and mix well. The solution will become cloudy upon mixing.
14. Pour the PBST off the membrane and add the prepared substrate solution. Incubate for 5–30 minutes or until the desired level of signal is attained.
15. Pour off the substrate and wash the membrane in ddH_2O for 15 minutes.
16. Dry the membrane and store or document.

Section 3
Alternative Protocol
(For kits 170-8238/39/40)

An alternative, and potentially more sensitive amplification, may be accomplished by substituting a biotinylated secondary antibody for the HRP-linked secondary antibody. There is an additional incubation in streptavidin-HRP that precedes incubation in the BAR solution. The kit has sufficient streptavidin-HRP to carry out detection over 2,500 cm^2 of membrane, even if you use a biotinylated secondary antibody.

This approach entails two rounds of amplification, so background can be a significant problem and it may not be trivial to work out the best conditions. Background is affected by the dilution of primary antibody, secondary antibody, the BAR and the streptavidin-HRP. It may be necessary to further dilute one or more of these four reagents.

A brief protocol is outlined below.

Blotting and amplification.

1. Transfer protein to nitrocellulose membrane by electrophoretic blotting, dot blotting or microfiltration. Allow membrane to air dry.
2. Wet membrane in PBST and then wash 2x for 5 minutes each time in PBST.
3. Block membrane in 3% blocker for 1 hour.
4. Wash 2x with PBST for 3–5 minutes.
5. Incubate in appropriately diluted primary antibody for one hour.
6. Wash 2x with PBST for 5 minutes each time.
7. Incubate in 1:3,000–1:10,000 dilution of biotinylated goat anti-rabbit IgG secondary antibody (catalog number 170-6401) for 1 hour.
8. Wash 2x with PBST for 5 minutes each time.
9. Incubate in 1:1,000 dilution of streptavidin-HRP for 30 minutes.

10. Wash 2x in PBST for 5 minutes each time.
11. Incubate membrane in diluted BAR for 10 minutes.
12. Wash 2–4x in 20% DMSO/PBST for 5 minutes each time.
13. Wash 1–2x in PBST for 5 minutes each time.
14. Incubate membrane in diluted streptavidin-HRP for 30 minutes.
15. Wash 2x in PBST for 5 minutes each time.

Colorimetric Detection

16. Mix one part Opti-4CN diluent concentrate with nine parts ddH_2O. Prepare 0.25 ml per cm^2 of membrane.
17. Add 0.2 ml of Opti-4CN substrate per 10 mls of diluent. Mix well and pour onto membrane.
18. Incubate membrane with gentle agitation in the substrate for up to 30 minutes or until the desired level of sensitivity is attained.
19. Wash the membrane in ddH_2O for 15 minutes.
20. Document or store membrane.

Section 4
Troubleshooting Guide

Problem	Probable Cause	Recommended Solution
1. No reaction or weak signal.	a. Exposure time was too short.	i. Increase the exposure time.
	b. Primary antibody solution is inactive or non-saturating.	i. Store the antibody solution at the proper temperature. Avoid bacterial contamination, heat inactivation, and repeated freeze-thaw cycles.
		ii. Antibody titer was too low. Increase the concentration of the antibody used in the assay.
		iii. Tween-20 may affect the reactivity of some antibodies. Eliminate Tween-20 from the assay (except the wash after the blocking step).

Troubleshooting Guide *(continued)*

Problem	Probable Cause	Recommended Solution
	c. Conjugate is inactive.	i. Store the conjugate at the proper temperature. Avoid repeated freeze-thaw cycles. ii. The concentration of the conjugate was non-saturating. Increase the concentration of the conjugate used in the assay. iii. Conjugate may be contaminated, causing inactivation of the antibody or enzyme. Tap water may cause inactivation; use only distilled, deionized water to prepare all solutions.

Troubleshooting Guide *(continued)*

Problem	Probable Cause	Recommended Solution
	d. Little or no antigen is bound to the membrane.	i. Tween-20 may wash bound antigen from the membrane. Eliminate Tween-20 from the assay (except the wash after the blocking step). ii. Transfer of protein onto the membrane was incomplete. Stain gel to assure transfer of protein. Use Prestained Standards to monitor transfer efficiency. Consult the appropriate instrument manual for proper procedures and recommendations.

Troubleshooting Guide *(continued)*

Problem	Probable Cause	Recommended Solution
	e. Primary antibody is not specific or does not recognize denatured antigens (common with monoclonals).	i. Loss of reac tivity may have occurred during elec-trophoretic transfer. Pre-test the reactiv-ity of the anti-body against the antigen by a dot blot.
2. High background.	a. Exposure time was too long.	i. Decrease exposure time.
	b. PBST washes after transfer were omit-ted or insufficient.	i. The washes are critical to reduce spotted or blotchy background development.
	c. Blocking was insuf-ficient.	i. Increase the time of the blocking step and/or the con-centration of blocker used.

Troubleshooting Guide *(continued)*

Problem	Probable Cause	Recommended Solution
	d. Wash stringency was insufficient.	i. Tween-20 is necessary in wash steps to reduce back-ground. The concentration can be increased up to 0.3% if back-ground persists.
		ii. Increase the number and length of washes.
	e. Second antibody conjugate was used at an excessive con-centration.	i. Use the recom-mended dilu tion,or deter-mine the opti-mal dilution experimentally.
	f. Contamination occurred during transfer.	i. Refer to the instrument instruction manual for rec-ommendations.
	g. Insufficient number of DMSO/PBST washes.	i. Increase number of wash steps following BAR.

Troubleshooting Guide *(continued)*

Problem	Probable Cause	Recommended Solution
	h. BAR concentration was too high.	i. Dilute BAR further.
	i. Streptavidin-HRP concentration was too high	i. Dilute Streptavidin-HRP further.

Section 5
Ordering Information

Catalog Number	Product Description
Opti-4CN Substrate and Detection Kits	
170-8235	**Opti-4CN Substrate Kit**
170-8236	**Opti-4CN Detection Kit, Goat-anti-Rabbit**
170-8237	**Opti-4CN Detection Kit, Goat-anti-Mouse**
170-8238	**Amplified Opti-4CN Substrate Kit**
170-8239	**Amplified Opti-4CN Detection Kit, Goat-anti-Rabbit**
170-8240	**Amplified Opti-4CN Detection Kit, Goat-anti-Mouse**
AmpLight™ Western Detection Kits	
170-8232	**AmpLight Fluorescent Western Detection Kit**
170-8234	**AmpLight Chemiluminescent Western Detection Kit**

Catalog Number	Product Description
Blotting Grade Conjugates	
170-6515	**Goat Anti-Rabbit IgG-HRP**
170-6516	**Goat Anti-Mouse IgG-HRP**
172-1050	**Goat Anti-Human IgG-HRP**
170-6401	**Biotinylated Goat Anti-Rabbit IgG**
Pure Nitrocellulose Membranes (0.45 micron)	
162-0145	**Sheets**, 7 x 8.4 cm, 10
162-0117	**Sheets**, 9 x 12 cm, 10
162-0114	**Sheets**, 15 x 9.2 cm, 10
162-0116	**Sheets**, 15 x 15, 10
162-0113	**Sheets**, 20 x 20 cm, 5
162-0115	**Roll**, 33 cm x 3 m, 1
Pure Nitrocellulose Membranes (0.2 micron)	
162-0146	**Sheets**, 7 x 8.4 cm, 10
162-0147	**Sheets**, 13.5 x 16.5 cm, 10
162-0150	**Sheets**, 20 x 20 cm, 5
162-0112	**Roll**, 33 cm x 3 m, 1
Supported Nitrocellulose Membranes (0.45 micron)	
162-0090	**Sheets**, 7 x 8.4 cm, 10
162-0091	**Sheets**, 10 x 15 cm, 10
162-0092	**Sheets**, 15 x 15, 10
162-0093	**Sheets**, 20 x 20 cm, 10
162-0094	**Roll**, 33 cm x 3 m, 1

Catalog Number	Product Description
Supported Nitrocellulose Membranes (0.2 micron)	
162-0095	**Sheets**, 7 x 8.4 cm, 10
162-0096	**Sheets**, 15 x 15 cm, 10
162-0097	**Roll**, 33 cm x 3 m, 1
PVDF Membranes (0.2 micron)	
162-0186	**Sheets**, 7 x 8.4 cm, 10
162-0180	**Sheets**, 10 x 15 cm, 10
162-0181	**Sheets**, 15 x 15, 10
162-0182	**Sheets**, 20 x 20 cm, 10
162-0185	**Sheets**, 20 x 20 cm, 3
162-0184	**Roll**, 24 cm x 3.3 m, 1
Miscellaneous	
170-6531	**Tween-20**, 100 ml