Immun-Blot® Kit for Glycoprotein Detection

Instruction Manual

Catalog Number
170-6490

For Technical Service
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(1-800-424-6723)

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Section 1
Preparation

1.1 Introduction

The Immun-Blot kit for glycoprotein detection is optimized for the positive detection of carbohydrate structures in glycoprotein molecules. The kit uses a specific carbohydrate oxidation reaction to label the carbohydrate with biotin and subsequent detection using streptavidin-alkaline phosphatase and color development reagents. Labeling can be performed either on glycoproteins in solution or after they are immobilized onto a membrane support. The kit may also be used to determine whether the non-reducing terminal monosaccharide of the glycan is sialic acid. Sialic acid can be selectively oxidized with a mild periodate treatment at 0 °C and detected with the same biotin-streptavidin labeling system.

Four protocols are included in this manual (Table 1) total carbohydrate labeling on a membrane (Protocol 1A), sialic acid labeling on a membrane (Protocol 1B), total carbohydrate labeling in solution (Protocol 2A), and sialic acid labeling in solution (Protocol 2B).

Table 1. Labeling Protocols

	Total Carbohydrate Labeling	Sialic Acid Labeling
Membrane Labeling	Protocol 1A	Protocol 1B
Solution Labeling	Protocol 2A	Protocol 2B

1.2 Safety Instructions

Read the entire instruction manual before beginning the assay.

1. Wear gloves and protective clothing, such as a laboratory coat and eye protection, when preparing and working with the solutions in the assay. DMF and BCIP can cause skin irritation, and contact should be avoided. In case of contact, immediately flush the skin or eyes with copious amounts of water for at least 15 minutes, and remove contaminated clothing.
2. Work in a well-ventilated area. Avoid inhalation of vapors when handling solutions containing DMF and BCIP.
3. Do not mouth-pipette any solutions.

1.3 Solution and Reagent Preparation

Solution Preparation

The following solutions are not supplied in the kit and must be prepared prior to use. Solutions 1-5 are required for all four protocols (1A, 1B, 2A, and 2B) while solutions 6 and 7 are required for protocols 1A and 1B only. Store all solutions at 4 °C (add 0.01% NaN_3 for long term storage).

Solutions

Description	Reagents/Quantities		Directions
1. TBS (50 mM Tris, 27 mM sodium chloride, pH 7.2)	6.05 g 1.6 g	Tris NaCl	Dissolve reagents in 900 ml ddH_2O. Adjust pH to 7.2 with1 N HCl. Q.S. to 1,000 ml with ddH_2O.

Description	Reagents/Quantities		Directions
2. 200 mM Sodium Acetate Buffer, pH 5.5	440 ml 60 ml	200 mM sodium acetate (12.0 g sodium acetate trihydrate in 440 ml ddH_2O) 200 mM acetic acid (690 µl glacial acetic acid in 60 ml ddH_2O)	Combine stock solutions and check pH is 5.5.
3. 4x Sample Buffer	250 µl 80 mg 400 µl 80 µl 200 µl 70 µl	1.0 M Tris pH 6.8 SDS Glycerol 0.5% bromo-phenol blue ß-mercapto-ethanol ddH_2O	Combine reagents and mix well. For optimal use prepare fresh each time.
4. Color Develop-ment Buffer	1.21 g 1.01 g 0.58 g 90 ml	Tris $MgCl_2 \cdot 6H_2O$ NaCl ddH_2O	Dissolve reagents in 90 ml ddH_2O. Adjust pH to 9.5 with 0.1 N HCl and Q.S. to 100 ml total volume with ddH_2O.
5. DMF	3.0 ml	Dimethylfor-mamide	Ready to use.
6. PBS (9 mM sodium phosphate, 27 mM sodium chloride, pH 7.2)	575 mg 100 mg 800 mg	Na_2HPO_4 NaH_2PO_4 NaCl	Dissolve reagents in 500 ml ddH_2O.
7. 100 mM Sodium Acetate/5 mM EDTA	1.14 g 300 ml	EDTA (tetra sodium salt) 200 mM sodium acetate, pH 5.5 (solution 2)	Dissolve EDTA in 300 ml of solution 2. Q.S. to 600 ml total volume with ddH_2O.

Reagent Preparation, Stability and Storage

Unused kit reagents should be stored at 4 °C prior to use (shelf life is 1 year). Prepare kit reagents prior to performing detection protocols and store as indicated. Subsequent protocols assume that each kit reagent has been reconstituted as indicated below.

Reagent	Contents	Reagent Preparation	Storage/Shelf Life After Preparation
A	Periodate Protocol 1	Dissolve 21.4 mg of vial A in 10 ml sodium acetate/ EDTA (solution 7) to make a 10 mM solution.	Prepare just prior to use. Light sensitive reagent.
B	Hydrazide	Dissolve vial B contents in 500 µl DMF (solution 5).	3 months at 4 °C.
C	Blocking Reagent	Dissolve 0.5 g of vial C in 100 ml TBS (solution 1); if necessary heat at 60 °C for 45 minutes to dissolve.	3 months at 4 °C.
D	Streptavidin-Alkaline Phosphatase	Ready to use.	3 months at 4 °C. Do not freeze.
E	NBT	Add 1.3 ml 70% (v/v) DMF (solution 5) to the contents of vial E and dissolve.	3 months at -20 °C. Light sensitive reagent.
F	BCIP	Add 1 ml DMF (solution 5) to the contents of vial F and dissolve.	3 months at -20 °C. Light sensitive reagent.
G	Periodate Protocol 2	Add 1.5 ml ddH_2O to the contents of vial G and dissolve.	1 month at 4 °C. Light sensitive reagent.

Reagent	Contents	Reagent Preparation	Storage/Shelf Life After Preparation
H	Bisulphite	Dissolve one tablet from vial H in 4 ml of 200 mM sodium acetate buffer (solution 2). Dilute 0.1 ml of this solution in 1 ml of sodium acetate buffer (solution 2).	1 day at 4 °C.
I	Ovalbumin (1 mg/ml)	Add 0.5 ml ddH_2O to the contents of vial I.	3 months at 4/-20 °C. Avoid repeated freeze-thaw cycles.
J	Biotinylated Markers	Just prior to use add 50 µl of 1X sample buffer. (4X sample buffer diluted 1:4 with H_2O.)	3 months at 4/-20 °C. Avoid repeated freeze-thaw cycles.
K	Native Markers	Just prior to use add 50 µl of 1X sample buffer. (4X sample buffer diluted 1:4 with H_2O.)	3 months at 4/-20 °C. Avoid repeated freeze-thaw cycles.

1.4 Membrane Selection

A variety of membranes are available for immunoblotting applications. The physical properties and performance characteristics of a membrane should be evaluated in selecting the appropriate transfer conditions. Nitrocellulose, a general purpose membrane with high signal and low background, is recommended for use in this kit. If a stronger membrane is needed use supported nitrocellulose.

Section 2
Immun-Blot Assay

2.1 Protocol Selection

The Immun-Blot kit for glycoprotein detection may be used to label glycosylated proteins immobilized on a membrane (Protocol 1A) or to label glycosylated proteins in solution (Protocol 2A). Refer to protocol 1B and 2B for preferential labeling of sialic acid groups immobilized on a membrane or in solution respectively.

Note: Substances which have been found to interfere with the oxidation and labeling chemistry are listed in Table 2. Since the detection is based on the periodate oxidation of carbohydrate groups followed by biotinylation and SDS-PAGE/blotting, substances which interfere with these steps should be avoided. If any of these substances are present in the sample solution, the membrane labeling protocol (1) is recommended.

Table 2. Interfering Substances

Substances
2-mercaptoethanol
Ammonium ions
Dithiothreitol
Ethylene glycol
Galactose
Glucose
Glycerol
Sodium borohydride
Transition metal ions (*e.g.* Ca, Mn, Cu, Zn, etc.)
Tris
Urea

2.2 Membrane Labeling Protocols

Protocol 1A describes the procedure for total carbohydrate labeling on membranes. This procedure is useful for the analysis of multiple samples, provides molecular weight information, and is not susceptible to interference with substances in the sample or buffers. To preferentially label sialic acid use protocol 1B.

Total Carbohydrate Detection on a Membrane (Protocol 1A)

1. Immobilize the protein sample onto the membrane by performing either a dot blot manually or with the Bio-Dot® or Bio-Dot SF microfiltration apparatus, or by performing SDS-PAGE with the Mini-PRO-

TEAN® II cell followed by a Western blot transfer with the Trans-Blot®, Trans-Blot SD, or Mini Trans-Blot® cell.

2. Following transfer, wash the membrane with 10 ml PBS (solution 6) at room temperature for 10 minutes with gentle agitation. **Do not use TBS as Tris will interfere with the subsequent biotinylation reactions.**
3. Immerse the membrane in 10 ml of 10 mM sodium periodate in sodium acetate/EDTA buffer (reagent A). Incubate in the dark (cover dish with aluminum foil) at room temperature for 20 minutes with gentle agitation.
4. Wash the membrane 3 times with 10 ml of PBS (solution 6) at room temperature for 10 minutes with gentle agitation. Use fresh buffer each time.
5. Prepare the biotinylation solution immediately before use by adding 2 µl hydrazide solution (reagent B) to 10 ml sodium acetate/EDTA buffer (solution 7). Immerse the membrane in this solution and incubate for 60 minutes at room temperature with gentle agitation.
6. Wash the membrane 3 times with 10 ml of TBS (solution 1) at room temperature for 10 minutes with gentle agitation. Use fresh buffer each time. (Note: TBS will no longer interfere with the process and may be used in subsequent steps.)
7. Immerse the membrane in 10 ml of blocking solution (reagent C). Incubate for at least 30 minutes at room temperature with gentle agitation or overnight at 4 °C.
8. Wash the membrane 3 times with 10 ml of TBS (solution 1) at room temperature for 10 minutes with gentle agitation. Use fresh buffer each time.
9. Prepare the conjugate solution by adding 5 µl Streptavidin-Alkaline Phosphatase conjugate (reagent D) to 10 ml TBS (solution 1). Immerse the membrane in this solution and incubate for 60 minutes at room temperature with gentle agitation.
10. Wash the membrane 3 times with 10 ml of TBS (solution 1) at room temperature for 10 minutes with gentle agitation. Use fresh buffer each time.
11. Prepare the color development solution immediately before use. Add 50 µl NBT (reagent E) and 37.5 µl BCIP (reagent F) to 10 ml of color development buffer (solution 4) and mix well. Immerse the membrane in the color development solution at room temperature **without agitation**. Develop to desired intensity (3-60 minutes).
12. To stop the development, rinse the membrane several times in ddH_2O and allow membrane to air dry.

Sialic Acid Labeling on a Membrane (Protocol 1B)

1. Immobilize the protein sample onto the membrane by performing either a dot blot manually or with the Bio-Dot or Bio-Dot SF microfiltration apparatus, or by performing SDS-PAGE with the or Mini-PROTEAN II cell followed by a Western blot transfer

with the Trans-Blot, Trans-Blot SD, or Mini Trans-Blot cell.

2. Following transfer, wash the membrane in ice-cold PBS (solution 6) for 10 minutes at 4 °C with gentle agitation. **Do not use TBS as Tris will interfere with the subsequent biotinylation reactions.**
3. Prepare 1 mM sodium periodate by adding 1 ml of 10 mM sodium periodate (reagent A) to 9 ml sodium acetate/EDTA buffer (solution 7). Immerse the membrane in 10 ml of this solution and incubate in the dark (cover dish with aluminum foil) at 4 °C for 20 minutes with gentle agitation.
4. Wash the membrane 3 times with 10 ml of PBS (solution 6) at 4 °C for 10 minutes with gentle agitation. Use fresh buffer each time.
5. Prepare the biotinylation solution immediately before use by adding 2 µl hydrazide solution (reagent B) to 10 ml sodium acetate/EDTA buffer (solution 7). Immerse the membrane in this solution and incubate for 60 minutes at room temperature with gentle agitation.
6. Wash the membrane 3 times with 10 ml of TBS (solution 1) at room temperature for 10 minutes with gentle agitation. Use fresh buffer each time. (Note: TBS will no longer interfere with the process and may be used in subsequent steps.)
7. Immerse the membrane in 10 ml of blocking solution (reagent C). Incubate for at least 30 minutes at room temperature with gentle agitation or overnight at 4 °C.

8. Wash the membrane 3 times with 10 ml of TBS (solution 1) at room temperature for 10 minutes with gentle agitation. Use fresh buffer each time.
9. Prepare the conjugate solution by adding 5 µl Streptavidin-Alkaline Phosphatase conjugate (reagent D) to 10 ml TBS (solution 1). Immerse the membrane in this solution and incubate for 60 minutes at room temperature with gentle agitation.
10. Wash the membrane 3 times with 10 ml of TBS (solution 1) at room temperature for 10 minutes with gentle agitation. Use fresh buffer each time.
11. Prepare the color development solution immediately before use. Add 50 µl NBT (reagent E) and 37.5 µl BCIP (reagent F) to 10 ml of color development buffer (solution 4) and mix well. Immerse the membrane in the color development solution at room temperature **without agitation**. Develop to desired intensity (3-60 minutes).
12. To stop the development, rinse the membrane several times in ddH_2O and allow membrane to air dry.

2.3 Solution Labeling Protocols

Protocol 2A describes the procedure for total carbohydrate labeling in solution. This procedure is more sensitive since all generated aldehydes are available for biotinylation. This method also exhibits lower background since the biotinylation reaction occurs in solution and the membrane is not incubated with biotin. However, this method is also more susceptible to interference or a false positive reaction due to the possibility of contaminating substances contained in the sample

buffer. In addition to the substances listed in Table 2, the following substances (Table 3) can interfere with solution labeling protocols (protocols 2A & 2B) if the maximum allowable concentration is exceeded. To preferentially label sialic acid use protocol 2B.

Table 3. Substances That Can Interfere with Solution Labeling Protocols

Substance	Maximum Allowable Concentration
Acetonitrile	50% w/v
Ammonium sulphate	1M
Copper (II) acetate	5 mM
Dimethylformamide	50% w/v
Ethylene glycol	50% v/v
Formaldehyde	0.5% v/v
Glutaraldehyde	0.5% v/v
Trichloroacetic acid	5% w/v
Tris/HCl, pH 7.4	50 mM
Urea	3M

Sample Preparation

The sample should be in 100 mM sodium acetate, pH 5.5 buffer (solution 2, diluted 1:1). Additionally, any buffer contained in a liquid sample should not interfere with the sodium acetate buffer's ability to bring the final pH to between 5 and 6.

Solid sample: Dissolve 0.01 - 20 µg of sample in 20 µl of 100 mM sodium acetate, pH 5.5 (solution 2, diluted 1:1).

Liquid sample: Dilute 10 µl of sample 1:1 in 200 mM sodium acetate, pH 5.5 (solution 2).

Total Carbohydrate Labeling in Solution (Protocol 2A)

1. Aliquot 20 µl of prepared sample into a polypropylene microcentrifuge tube.
2. Add 10 µl of periodate solution (reagent G), mix and incubate at room temperature for 20 minutes in the dark (cover tube with aluminum foil).
3. Add 10 µl of bisulphite solution (reagent H), mix, and incubate at room temperature for 5 minutes.
4. Add 5 µl of hydrazide solution (reagent B), mix, and incubate at room temperature for 60 minutes.
5. Immobilize protein sample onto the membrane by performing either a dot blot manually or using the Bio-Dot or Bio-Dot SF microfiltration apparatus, or by performing SDS-PAGE electrophoresis with the Mini PROTEAN II cell followed by a Western blot transfer with the Trans-Blot, Trans-Blot SD or Mini Trans-Blot cell. (For SDS-PAGE sample preparation add 15 µl of 4x sample buffer (solution 3) to the reaction solution and heat for 2 minutes at 100 °C.)
6. Immerse the membrane in 10 ml of blocking solution (reagent C). Incubate for at least 30 minutes at room temperature with gentle agitation or overnight at 4 °C.

7. Wash the membrane 3 times with 10 ml of TBS (solution 1) at room temperature for 10 minutes with gentle agitation. Use fresh buffer each time.
8. Prepare the conjugate solution by adding 5 µl Streptavidin-Alkaline Phosphatase conjugate (reagent D) to 10 ml TBS (solution 1). Immerse the membrane in this solution and incubate for 60 minutes at room temperature with gentle agitation.
9. Wash the membrane 3 times with 10 ml of TBS (solution 1) at room temperature for 10 minutes with gentle agitation. Use fresh buffer each time.
10. Prepare the color development solution immediately before use. Add 50 µl NBT (reagent E) and 37.5 µl BCIP (reagent F) to 10 ml of color development buffer (solution 4) and mix well. Immerse the membrane in the color development solution at room temperature **without agitation**. Develop to desired intensity (3-60 minutes).
11. To stop the development, rinse the membrane several times in ddH_2O and allow membrane to air dry.

Sialic Acid Labeling in Solution (Protocol 2B)

1. Aliquot 20 µl of prepared sample to a polypropylene microcentrifuge tube and cool to 0 °C by placing on wet ice.
2. Add 2 µl periodate solution (reagent G) to 18 µl ddH_2O. Bring solution to 0 °C by placing on wet ice.
3. Add 10 µl of the diluted periodate solution (step 2) to the 20 µl sample (step 1). Mix well and incubate for 20 minutes at 0 °C in the dark (cover tube with aluminum foil).

4. Add 10 µl of bisulphite solution (reagent H), mix and incubate at room temperature for 5 minutes.
5. Add 5 µl of hydrazide solution (reagent B), mix and incubate at room temperature for 60 minutes.
6. Immobilize protein sample onto the membrane by performing either a dot blot manually or using the Bio-Dot or Bio-Dot SF microfiltration apparatus, or by performing SDS-PAGE electrophoresis with the Mini-PROTEAN II cell followed by a Western blot transfer with the Trans-Blot, Trans-Blot SD, or the Mini Trans-Blot cell. (For SDS-PAGE sample preparation add 15 µl of 4x sample buffer (solution 3) to the reaction solution and heat for 2 minutes at 100 °C.)
7. Immerse the membrane in 10 ml of blocking solution (reagent C). Incubate for at least 30 minutes at room temperature with gentle agitation or overnight at 4 °C.
8. Wash the membrane 3 times with 10 ml of TBS (solution 1) at room temperature for 10 minutes with gentle agitation. Use fresh buffer each time.
9. Prepare the conjugate solution by adding 5 µl Streptavidin-Alkaline Phosphatase conjugate (reagent D) to 10 ml TBS (solution 1). Immerse the membrane in this solution and incubate for 60 minutes at room temperature with gentle agitation.
10. Wash the membrane 3 times with 10 ml of TBS (solution 1) at room temperature for 10 minutes with gentle agitation. Use fresh buffer each time.
11. Prepare the color development solution immediately before use. Add 50 µl NBT (reagent E) and 37.5 µl

BCIP (reagent F) to 10 ml of color development buffer (solution 4) and mix well. Immerse the membrane in the color development solution at room temperature **without agitation**. Develop to desired intensity (3-60 minutes).

12. To stop the development, rinse the membrane several times in ddH_2O and allow membrane to air dry.

2.4 Interpretation of Assay Results

Positive Results - Signal Detected

Signal detection of the sample indicates the presence of carbohydrate in the sample. A negative control should be employed to verify that the positive result obtained is due to a carbohydrate moiety and is not a false positive due to an interfering substance. Repeat the assay using a negative control (a reaction in which the periodate reagent is replaced with water). If the negative control assay is positive then an interfering substance is present. If the negative control assay is negative and the sample is positive then a carbohydrate moiety is present in the sample.

Negative Results - No Signal Detected

No signal detection of the sample indicates that carbohydrate is not present in the sample. A positive control should be employed to verify that the negative result obtained is not due to the presence of an interfering substance. Repeat the experiment using the Ovalbumin positive control. If the positive control assay is negative then an interfering substance is present. If the positive control assay is positive and the sample is negative, then the sample does not contain a carbohydrate moiety.

Section 3
Troubleshooting Guide

Problem	Probable Cause	Recommended Solution
1. No reaction or weak color development	A. Color development solution is inactive.	A1. Color development reagent (E&F) must be stored at the proper temperature (see Section 1.3)
		A2. Avoid bacterial contamination of the color development buffer by storage at 4 °C.
		A3. Tap water can inactivate the color development solution. Use only distilled, deionized water to prepare the reagents.
	B. Conjugate is inactive.	B1. Conjugate is improperly stored. Store at 4 °C. Avoid heat inactivation. Do not freeze.
		B2. The concentration of conjugate is non-saturating. Increase the conjugate concentration used in the assay.
		B3. Conjugate may be contaminated causing inactivation. Replace conjugate reagent.
		B4. Tap water may cause inactivation. Use only distilled, deionized water.

Troubleshooting Guide (continued)

Problem	Probable Cause	Recommended Solution
	C. Little or no protein is bound to the membrane.	C1. Transfer of protein onto the membrane was incomplete. Stain the gel to assure transfer of protein. Use prestained standards to monitor transfer efficiency. Consult blotting apparatus manual for proper electrophoretic transfer procedures.
	D. Insufficient protein present.	D1. Increase sample protein concentration.
	E. Interfering substance.	E1. Repeat experiment with Ovalbumin control to determine the presence of an interfering substance. If interference is detected use Protocol 1(membrane labeling).
	F. Sample does not contain carbohydrate or the carbohydrate component is lacking the periodate-sensitive adjacent hydroxyl groups.	
2. High background	A. Blocking is insufficient.	A1. Increase the length of time the membrane is incubated in the blocking reagent.
	B. Blocking reagent is old.	B1. Prepare new blocking reagent.
	C. Nitrocellulose membrane is left in the development solution too long.	C1. Remove the membrane when the reaction appears to be complete. If precipitate in the color development solution appears, decant the solution and use fresh reagent.

Troubleshooting Guide (continued)

Problem	Probable Cause	Recommended Solution
	D. Conjugate is used at an excessive concentration.	D1. Use the recommended 1:2,000 dilution.
	E. Contamination occurred during electrophoresis or transfer.	E1. Filter all gel and transfer reagents through a nitrocellulose filter to remove any contaminating proteins.
		E2. Consult blotting apparatus manual.
	F. Use of poor quality, mixed ester nitrocellulose.	F1. Use pure nitrocellulose from Bio-Rad.
3. False positive reaction	A. Contamination during solution labeling protocol (2) occurred.	A1. Use protocol 1 (membrane labeling).

Section 4
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Section 5
Product Information

Catalog Number	Production Description

Detection Kit

170-6490 **Immun-Blot Kit for Glycoprotein Detection**

Blotting Membranes
Nitrocellulose Membrane (0.45 µm)

162-0115 **Roll,** 33 cm x 3 m, 1
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 cm, 10
162-0113 **Sheets,** 20 x 20 cm, 5
162-0148 **Sheets,** 11.5 x 16 cm, 10

Nitrocellulose Membrane (0.2 µm)

162-0112 **Roll,** 33 cm x 3 m, 1
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

Supported Nitrocellulose Membrane (0.45 µm)

162-0090 **Sheets,** 7 x 8.4 cm, 10

Catalog Number	Production Description

162-0091 **Sheets,** 10 x 15 cm, 10
162-0092 **Sheets,** 15 x 15 cm, 10
162-0093 **Sheets,** 20 x 20 cm, 5
162-0094 **Roll,** 30 cm x 3m, 1

Supported Nitrocellulose Membrane (0.2 µm)

162-0095 **Sheets,** 7 x 8.4 cm, 10
162-0096 **Sheets,** 15 x 15 cm, 10
162-0097 **Roll,** 30 cm x 3m, 1

PVDF Membrane (0.2 µm)

162-0180 **Sheets,** 10 x 15 cm, 10
162-0181 **Sheets,** 15 x 15 cm, 10
162-0182 **Sheets,** 20 x 20 cm, 10
162-0184 **Roll,** 24 cm x 3.3 m, 1
162-0185 **Sheets,** 20 cm x 20 cm, 3
162-0186 **Sheets,** 7 x 8.4 cm, 10

Ready Gels, 10 gel pack

161-0900 **Ready Gel,** 7.5% resolving gel, 4% stacking gel, 0.375 M Tris-HCl, 10 well comb
161-0907 **Ready Gel,** 10% resolving gel, 4% stacking gel, 0.375 M Tris-HCl, 10 well comb
161-0901 **Ready Gel,** 12% resolving gel, 4% stacking gel, 0.375 M Tris-HCl, 10 well comb
161-0909 **Ready Gel,** 12% resolving gel, 4% stacking gel, 0.375 M Tris-HCl, 2 well prep comb
161-0908 **Ready Gel,** 15% resolving gel, 4% stacking gel, 0.375 M Tris-HCl, 10 well comb
161-0902 **Ready Gel,** 4-15% gradient gel, 0.375 M Tris-HCl, 10 well comb

Catalog Number	Production Description
161-0903	**Ready Gel,** 4-20% gradient gel, 0.375 M Tris-HCl, 10 well comb
161-0906	**Ready Gel,** 10-20% gradient gel, 4% stacking gel, 0.375 M Tris-HCl, 10 well comb161-0922 **Ready Gel,** 16.5% Tris Tricine gel, 4% stacking gel, 10 well comb
161-0923	**Ready Gel,** 10-20% Tris Tricine gel, 4% stacking gel, 10 well comb

Electrophoresis Buffer Reagents

161-0716	**Tris,** 500 g
161-0719	**Tris,** 1 kg
161-0728	**EDTA,** 100 g
161-0729	**EDTA,** 500 g
161-0710	**2-mercaptoethanol,** 25 ml
161-0300	**SDS,** 25 g
161-0301	**SDS,** 100 g
161-0302	**SDS,** 1 kg
161-0416	**SDS Solution 10% (w/v),** 250 ml
161-0418	**SDS Solution 20% (w/v),** 1000 ml
161-0404	**Bromophenol Blue,** 10 g

Premixed Buffers

161-0732	**10x Tris/Glycine/SDS,** 1 L
161-0755	**10x Tris/Glycine/SDS,** 6 x 1 L
161-0734	**10x Tris/Glycine,** 1 L
161-0757	**10x Tris/Glycine,** 6 x 1 L
161-0744	**10x Tris/Tricine/SDS,** 1 L
161-0760	**10x Tris/Tricine/SDS,** 6 x 1 L

Catalog Number	Production Description

Premixed Sample Buffers

161-0737	**Laemmli Sample Buffer,** 30 ml
161-0738	**Native Sample Buffer,** 30 ml
161-0739	**Tricine Sample Buffer,** 30 ml

Section 6
Technical Support

If you require additional technical assistance contact your local Bio-Rad representative, or, in the U.S., dial 1-800 4BIORAD (1-800-424-6723) and press 2 for the technical service department.

Section 7
Appendix

7.1 Molecular Weight Markers

Biotinylated Markers	Molecular Weight
Phosphorylase B	97,400
Catalase	58,100
Alcohol dehydrogenase	39,800
Carbonic anhydrase	29,000
Trypsin inhibitor	20,100
Lysozyme	14,300

Native Markers	Molecular Weight
Myosin	205,000
ß-galactosidase	116,000
Phosphorylase B	97,400
Bovine serum albumin	66,000
Ovalbumin	45,000
Carbonic anhydrase	29,000

Catalog Number	Production Description
Protein Standards	
161-0325	**Kaleidoscope Polypeptide Standards,** 500 µl
161-0324	**Kaleidoscope Prestained Standards,** 500 µl
161-0305	**Prestained SDS-PAGE Standards, low range,** 500 µl
161-0309	**Prestained SDS-PAGE Standards, high range,** 500 µl
161-0318	**Prestained SDS-PAGE Standards, broad range,** 500 µl
161-0306	**Biotinylated SDS-PAGE Standards, low range,** 250 µl
161-0311	**Biotinylated SDS-PAGE Standards, high range,** 250 µl
161-0319	**Biotinylated SDS-PAGE Standards, broad range,** 250 µl
Electrophoresis and Blotting Equipment	
165-2940	**Mini-PROTEAN II Cell**
170-3939	**Trans-Blot Cell with Plate Electrodes**
170-3910	**Trans-Blot Cell with Wire Electrodes**
170-3940	**Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell**
170-3930	**Mini Trans-Blot Electrophoretic Transfer Cell**

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4006042 Rev B