

Nonfat Dry Milk

250 grams



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rev. 10/24/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Description: Premium quality nonfat dry milk for use as a blocking agent during Western blotting to block non-specific antibody binding and reduce background signal.

Storage: Store at room temperature.

Companion Products:

Tris Buffered Saline with Tween 20 (TBST - 10X) #9997

BSA #9998

Prestained Protein Marker, Broad Range (Premixed Format) #7720

Biotinylated Protein Ladder Detection Pack #7727

20X LumiGLO® Reagent and 20X Peroxide #7003

Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071

Phototope®-HRP Western Blot Detection System, Anti-mouse IgG, HRP-linked Antibody #7072

Anti-rabbit IgG, HRP-linked Antibody #7074

Anti-biotin, HRP-linked Antibody #7075

Anti-mouse IgG, HRP-linked Antibody #7076

Anti-rat IgG, HRP-linked Antibody #7077

Western Immunoblotting Protocol (Primary Ab Incubation In Milk)

For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7072:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight marker (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.

Material Safety Data Sheet (MSDS) for Nonfat Dry Milk

rev. 10/24/08

I. Identification:

Product name: Nonfat Dry Milk**Product Catalog:** 9999

Manufacturer Supplier: Cell Signaling Technology
3 Trask Lane
Danvers, MA 01923 USA
1-978-867-2300 TEL
1-978-867-2400 FAX
1-978-578-6737 Emergency TEL

II. Composition/Information on Ingredients:

Substance Name: nonfat-dried milk from bovine
CAS#: none

III. Hazard Identification:

HMIS rating	NFPA Rating
Health: 0	Health: 0
Flammability: 0	Flammability: 0
Reactivity: 0	Reactivity: 0

IV. First Aid Measures:

Inhalation: Remove to fresh air. If breathing is difficult, get medical attention.**Ingestion:** If person is conscious wash out mouth with water. Get medical attention.**Skin exposure:** Wash skin with soap and water. If irritation develops or persists, get medical attention.**Eye exposure:** Immediately flush eyes water for at least 15 minutes lifting upper and lower lids. Get medical attention.

V. Fire Fighting Measures:

Flash Point: Not applicable**Autoignition Temperature:** Not applicable**Explosion:** Not considered not applicable.**Fire extinguishing media:** Water spray, dry chemical, appropriate foam, or carbon dioxide.**Firefighting:** Wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes.**Specific Hazard:** Emits toxic fumes under fire conditions.

VI. Accidental Release Measures: Use personal proactive equipment as indicated in section VIII. Sweep up, avoid raising dust, and transfer to a suitable waste container for disposal. Wash spill site after material has been picked up.

VII. Handling And Storage:

Handling: Use with adequate ventilation. Avoid contact with eyes and skin, wash thoroughly after handling.**Storage:** Store in tightly closed container in cool, dry place.

VIII. Exposure Controls/Personal:

Ventilation System: a system of local and/or general exhaust is recommended.**Skin Protection:** wear protective gloves and lab coat to minimize contact with skin.**Eye protection:** wear protective eyeglasses or chemical safety goggles. Maintain eye wash fountain and quick-drench facilities in work area.

IX. Physical And Chemical Properties:

Appearance:	off-white powder
Odor:	slight
pH	data not available
Boiling Point:	data not available
Melting Point:	data not available
Volatile Organic Compounds (VOC):	data not available
Solubility:	Soluble in water

X. Stability and Reactivity:

Stability: Stable**Conditions to avoid:** strong oxidizing agents.**Hazardous Decomposition Products:** data not available.**Hazardous polymerization:** data not available.

XI. Toxicological Information:

Routes of Exposure**Skin:** May cause skin irritation. May be harmful if absorbed through the skin.**Eye:** May cause eye irritation.**Inhalation:** May be irritating to mucous membranes and upper respiratory tract. May be harmful if inhaled.**Ingestion:** May be harmful if swallowed.**Signs and symptoms of exposure:** To the best of our knowledge the chemical, physical, and toxicological properties have not been thoroughly investigated.

XII. Ecological Information:

No data available.

XIII. Disposal Considerations:

Contact a licensed professional waste disposal service to dispose of this material. Observe all federal, state and local environmental regulations.

XIV. Transport Information:

D.O.T.**Proper Shipping Name:** None

This substance is considered non-hazardous for transport.

IATA**Proper Shipping Name:** None

This substance is considered non-hazardous for air transport.

XV. Regulatory Information:

US Regulatory Information:**SARA:** Not listed**Canada (WHMIS):** Not listed on DSL/NDL

XVI. Other Information:

This compound is sold only for research use by personnel familiar with chemicals and who are well trained in good laboratory habits, such as avoiding spills, keeping hands clean at all times and not rubbing eyes with hands while working in the laboratory. This product is not intended for drug, household, or other uses.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide for experienced personnel. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product. The burden of safe use of this material rests entirely with the user.

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