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OxyBlot™ Protein Standard

CATALOG NUMBER: S7151

LOT NUMBER:

QUANTITY: 150 µL

DESCRIPTION:

OxyBlot™ Protein Standard is composed of five proteins (phosphorylase B, BSA, ovalbumin, carbonic anhydrase, trypsin inhibitor) containing 1-3 dinitrophenylhydrazone (DNP) residues per protein. These attached DNP moieties permit the visualization of the proteins by the detection system of the OxyBlot™ Protein Oxidation Detection Kit.

Intended Use

OxyBlot™ Protein Standard serves as an internal control for the electrophoresis, western transfer and immunodetection steps of the OxyBlot™ procedure.

APPLICATIONS:

Material Provided:

Each vial contains 150 µL of OxyBlot™ Protein Standard, which is sufficient for 60 minigels.

Instructions for Use:

Warm the mixture to room temperature. (Since the mixture contains SDS that may precipitate during storage, it may be necessary to heat the stock solution to 50°C to redissolve the SDS). Combine 2.5 µL of the OxyBlot™ Protein Standard with 20 µL of 1X Gel Loading Buffer prior to loading onto the minigel. Do not heat prior to gel loading. Perform SDS-PAGE according to standard procedures.

NOTE: Since the proteins in the standard mixture already contain DNP residues, the protein standard should not be subjected to the derivatization reaction in the OxyBlot™ Protein Oxidation Detection Kit protocol.

In order to maintain consistency, the 2-Mercaptoethanol must be omitted from the 1X Gel Loading Buffer if a reducing agent was not used in the preparation of the experimental samples.

Reagent Preparation:

1X Gel Loading Buffer

To make 8 ml:

0.5 M Tris-HCl, pH 6.8	1.0 mL
Glycerol	0.8 mL
14.3 M 2-Mercaptoethanol (Reagent Grade)	0.1 mL
10% SDS	1.6 mL
0.05% Bromophenol Blue	0.32 mL
dH2O	4.18 mL

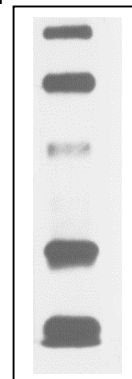
Divide into 1 mL aliquots and store at -15°C to -25°C.

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OxyBlot™ Protein Standard

Protein	Molecular Weight
Phosphorylase B	97,400
Bovine serum albumin	68,000
Ovalbumin	43,000
Carbonic anhydrase	29,000
Trypsin inhibitor	21,000



2.5 μ L of DNP-protein mixture was mixed with 20 μ L of 1X Gel Loading Buffer, separated on a 12% SDS polyacrylamide gel and electroblotted onto nitrocellulose membrane. The membrane was processed according to the procedure outlined in the OxyBlot™ Kit protocol, using reagents in the kit. The protein bands on the filter were detected using a chemiluminescent detection kit. The chemiluminescent signal was captured by a 15 second exposure on film.

OxyBlot™ is a trademark of Serologicals Company

KIT COMPONENTS: Each vial contains 150 μ L of OxyBlot™ Protein Standard, which is sufficient for 60 minigels.

STORAGE/HANDLING: Prior to first use, store at 4°C. After using for the first time, divide the remaining mixture among several tubes. Store the aliquots at -15°C to -25°C. Dividing the mixture into aliquots serves to protect the mixture from excessive freeze-thaw cycles.

For research use only; not for use as a diagnostic