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ProSieve® ProTrack™ Dual Color Protein Loading Buffer

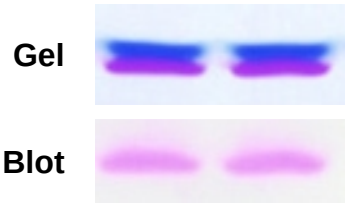
Instructions for Use

Introduction

The ProSieve® ProTrack™ Dual Color Protein Loading Buffer protects proteins from heat degradation during the sample preparation step, as well against pH changes during the SDS-PAGE run. Some proteins are sensitive to pH changes that result from temperature fluctuations during electrophoresis in Tris buffers. The optimized composition of the loading buffer prevents protein degradation during sample heating prior to SDS-PAGE[1] as well as during the electrophoresis run.

The loading buffer contains two tracking dyes: blue (bromophenol blue) for tracking the progress of electrophoresis and pink (pyronin Y) for monitoring of protein transfer to the membrane during Western blotting procedures. ProSieve® ProTrack™ Loading Buffer also contains SDS and DTT for complete disruption of all high-order protein structures.

Protein samples prepared with ProSieve® ProTrack™ Dual Color Protein Loading Buffer



Contents

Cat. No. 00193861
4X ProSieve® ProTrack™ Loading Buffer, 5 ml
(1000 applications @ 20ul)

Storage Buffer
0.25 M Tris-HCl, 1.6 mM EDTA (pH 8.5 at 25°C), 8% (w/v) SDS, 40% (w/v) glycerol, 0.04% (w/v) bromophenol blue, 0.024% (w/v) pyronin Y.

Store at Room Temperature

20X Reducing Agent (2 M DTT), 1 ml
Store at -20°C

Protocol

Thaw → Mix → Denature → Load

1. Thaw the Reducing Agent at room temperature. Dissolve precipitated solids in the Loading buffer (if any) at 37°C.
2. Gently mix the loading buffer and reducing agent to ensure that the solutions are homogeneous.
3. Add appropriate 20X Reducing Agent, ProSieve® ProTrack™ Loading Buffer and protein sample solution (protein sample in water) into clean microcentrifuge tube. Refer to table below for recommended volumes.
4. Heat samples at 100°C for 3-5 minutes.
5. Centrifuge briefly and apply directly to a SDS-polyacrylamide gel.

Recommended Volumes for Gel Loading

Gel Loading Volume	DTT*	Loading Dye	Sample Solution†
14 µl	0.7 µl	3.5 µl	9.8 µl
20 µl	1 µl	5 µl	14 µl
30 µl	1.5 µl	7.5 µl	21 µl
40 µl	2 µl	10 µl	28 µl

* For silver staining see Note.
† diH_2O may need to be added to samples to yield volume

Note

- For silver staining, DTT concentration in the sample should not exceed 50 mM. Higher DTT concentration in protein sample may cause streaking or yellowing of the gel. If initial protein sample contains >50 mM DTT, do not add Reducing Agent into the protein loading buffer.
- Due to presence of SDS, the loading buffer is not suitable for native polyacrylamide gel electrophoresis.
- Generally pyronin Y migrates faster than bromophenol blue. In higher percentage SDS-polyacrylamide gels (for example 15%) pyronin Y dye migrates slower than bromophenol blue.

Reference

1. Electrophoresis in practice, 4th edition, Westermeier, R., Wiley-VCH, 2005.

Product Safety

For details regarding product safety, see Material Safety Data Sheet (MSDS); call +1 (800) 638-8174 for extra copies of the MSDS. Emergency after hours, call collect +1 (303) 595-9048.

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