INSTRUCTIONS

Lane Marker Sample Buffers

39000 39001

0458.4

Number	Description
39000	**Lane Marker Reducing Sample Buffer (5X)**, 5mL, contains 0.3M Tris•HCl, 5% SDS, 50% glycerol, 100mM dithiothreitol (DTT) and proprietary pink tracking dye **Storage:** Upon receipt store at -20°C. Product is shipped with ice pack. DTT oxidizes with time, which reduces its ability to reduce disulfide bonds. Therefore, after thawing the reagent for its first use, aliquot the stock solution into single-use volumes and store at -20°C.
39001	**Lane Marker Non-Reducing Sample Buffer (5X)**, 5mL, contains 0.3M Tris•HCl, 5% SDS, 50% glycerol and proprietary pink tracking dye **Storage:** Upon receipt store at room temperature. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific Lane Marker Reducing and Non-Reducing Sample Buffers are convenient and ready to use for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. Formulated as 5X rather than the traditional 2X stocks, they enable a larger volume of sample to be loaded per well. Particularly unique in these buffers is the use of a bright pink tracking dye instead of the traditional bromophenol blue dye. This pink dye marks not only the dye front in the gel, but also transfers and permanently binds to nitrocellulose membranes. Consequently, the dye can be used to assess transfer efficiency and align cut membranes. The Reducing Sample Buffer contains DTT as the reducing agent, eliminating the repugnant odor associated with mercaptoethanol-containing buffers.

Lane Marker Sample Buffers are easy to use. Simply mix one part Sample Buffer with four parts protein sample, heat denature and load the gel sample wells. Visualize progress of the electrophoresis run by monitoring the location of the pink dye front. Lane Marker Sample Buffers may be used in denaturing gels of all kinds (including PhastGel® Systems) and are compatible with coomassie dye and silver staining techniques, as well as Western blotting procedures.

Procedure Summary

1. Equilibrate Lane Marker Sample Buffer to room temperature.
2. Mix one volume Sample Buffer with four volumes of protein sample (e.g., 5µL Sample Buffer + 20µL protein sample).
3. Boil sample for 3-5 minutes.
4. Cool sample to room temperature.
5. Load sample onto gel and perform the electrophoresis.
6. Stop electrophoresis when pink dye nears bottom of gel.

Procedure

1. Equilibrate Lane Marker Sample Buffer to room temperature. The bottle or tube containing Sample Buffer may be thawed quickly with warm water without harming the reagent.

   Note: Product will not fully dissolve to a homogeneous solution until it is at room temperature.
2. Mix one volume of Sample Buffer with four volumes of protein sample (e.g., 5µL Sample Buffer + 20µL protein sample). Failure to properly dilute sample buffer will cause a diffuse dye front.
3. Boil sample for 3-5 minutes to denature proteins.

4. Allow sample to cool to room temperature.
5. Apply sample onto the SDS-polyacrylamide gel and begin electrophoresis.
6. Stop electrophoresis when the pink dye front is approximately 0.5cm from the bottom of the gel.
7. Mark the top of the gel in the following manner (Optional):
   a. Disconnect the lid of the electrophoresis unit.
   b. Apply 2-5µL stock or diluted Lane Marker Sample Buffer to the appropriate sample wells.
   Note: To easily identify each lane, make an informative pattern with the Sample Buffer by applying it to particular lanes. For example, add the Sample Buffer to lanes 1-9, thereby distinguishing lane 1 from 10.
   c. Replace lid of electrophoresis unit and apply current for 1-2 minutes until Sample Buffer completely enters the gel.
   d. Stop electrophoresis.
8. Remove gel from electrophoresis unit. To stain gel, wash, fix and stain according to usual procedures. To transfer proteins and tracking dye to nitrocellulose membrane, equilibrate the gel in transfer buffer and proceed with usual methods.

Notes:

- The pink dye will not diffuse from gel, even if left in buffer for several hours before staining or transferring.
- The marker dye transfers and binds only to nitrocellulose. The dye does not transfer and bind to nylon or PVDF membranes.
- The pink dye autofluoresces and, therefore, might interfere with fluorescence detection applications.

Troubleshooting

Problem	Cause	Solution
Diffuse dye front	Sample Buffer too concentrated	Dilute Sample Buffer at least five-fold
No binding of Lane Marker dye to membrane upon transfer	PVDF and nylon membranes were used	Use nitrocellulose membrane

Related Thermo Scientific Products

28378 **BupH Tris-Glycine-SDS Buffer Packs**

28380 **BupH Tris-Glycine Transfer Buffer**

28398 **BupH Tris-HEPES-SDS Running Buffer Packs**

25200-44 **Precise™ Protein Gels**

PhastGel is a trademark of GE Healthcare Bio-Sciences AB LLC.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.