

DeStreak Rehydration Solution

Instructions for Use

Contents

5 × 3 mL DeStreak Rehydration Solution.
Short instruction sheet (Product code. 71502542).

Introduction

DeStreak Rehydration Solution is designed for the preparation of Immobiline™ DryStrip gels. During the first dimension run in Immobiline DryStrip, DeStreak Rehydration Solution transforms the protein thiol groups into stable disulfides and protects the disulfide groups from unspecific oxidation. This will reduce streak between spots in the pH range 7–9 and also simplify the spot pattern, as it reduces the number of spots caused by oxidation of proteins.

Sample preparation

Prepare the protein extract in sample buffer containing 20 mM reducing agents like dithiothreitol (DTT), β-mercaptoethanol or tris(2-carboxyethyl)-phosphine (TCEP). Using cup application the sample solution may contain up to 1 mg protein/mL. Using anodic paper bridge loading higher concentrations can be used. Dilute the original protein extract with sample solution.

Preparation of DeStreak Rehydration Solution

Note: Before use, equilibrate DeStreak Rehydration Solution at room temperature for 30 minutes. Shake the bottle to dissolve urea crystals.

DeStreak Rehydration Solution is delivered without IPG buffer. Before use, add 15 µL or 60 µL (0.5 or 2.0%) of the appropriate IPG buffer or Pharmalyte™ to 3 mL of DeStreak Rehydration Solution. Use 0.5% IPG Buffer in the Rehydration Solution when:

- IPGphor™ Regular Strip Holder is used for the first dimension.
- Horizontal gels are used in the second dimension.
- Using 10 kVolts in the Cup Loading Manifold.
- Immobiline DryStrip 7–11 NL and 3–11 NL are used. This will give high voltage and short run time in hours, which is essential for streak free results.

Use 2% IPG Buffer in the Rehydration Solution when the highest solubility of proteins and stability against salt is needed. However, this will give a higher conductivity and the highest voltage may not be reached.

Select an IPG buffer with the same pH interval as the Immobiline DryStrip being rehydrated, see [Table 1, on page 1](#)

Sample Application

The sample can be loaded in the rehydration solution or loaded after rehydration using a sample cup or anodic paper bridge.

With acidic pH intervals (3.5–5.6) we recommend rehydration loading or cathodic sample cup application. 100 µL sample may contain up to 20 mM reducing agents.

With neutral (4–7) and wide (3–10) pH intervals all sample application methods can be used, but sample specific limitations may exist. 100 µL sample may contain up to 10 mM reducing agents. With basic Immobiline DryStrip (pH intervals 6–11) we recommend anodic cup application or anodic paper bridge. 100 µL sample may contain up to 20 mM reducing agents.

Using rehydration loading on basic strips, the sample (the rehydration solution) may contain up to 1 mM reducing agent. This reducing power will be consumed during the rehydration step and early start of the run and thiols will be transferred to disulphides during the run.

Rehydrate the Immobiline DryStrip

Step	Action
1	Pipette the appropriate volume of prepared DeStreak Rehydration Solution to the Reswelling Tray, or the Regular Strip Holder as indicated in Table 1, on page 1 . Distribute the solution evenly over the strip length.
2	Carefully remove the cover foil from the Immobiline DryStrip, start from the anode (+end).
3	Carefully place the Immobiline DryStrip in the tray channel gel side down. Take care to distribute the rehydration solution evenly under the strip. Avoid trapping air bubbles under the strip.
4	Overlay the strip with Immobiline DryStrip Cover Fluid. Rehydrate for 10 to 20 hours.

Table 1. Volume of rehydration solution to Immobiline DryStrip of various lengths.

Immobiline DryStrip length (cm)	Rehydration volume /strip (µL)
7	125
11	200
13	250
18	340
24	450

Run

Run according to Short Instruction attached to the Immobiline DryStrip package or, Handbook 2–D Electrophoresis: Principles and Methods Cytiva (Product code 80642960).

Iso-electric points

At pH > 7, the isoelectric point of proteins may change due to the transfer of thiols or oxidation products to disulphides.

Technical data

Components: urea, thiourea, CHAPS, and DeStreak Reagent.
Storage: below -20°C.
Shelf life: See expiry date on package

Ordering information

Product	Quantity	Product code
DeStreak Reagent	1 mL	17600318
DeStreak Rehydration Solution	5 × 3 mL	17600319

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