DeStreak Reagent

Instructions for Use

Contents

1 mL DeStreak Reagent.

Short instruction sheet (Code no. 71502539).

Introduction

Unspecific oxidation of protein thiol groups occurs during 2–D electrophoresis of proteins, especially at pH > 7. In the resulting protein map this is seen as horizontal streak and extra spots.

Transforming the thiol groups into stable disulphide groups using DeStreak Reagent prevents unspecific oxidation. The preparation of Immobiline™ DryStrip with DeStreak Reagent will result in 2–D maps with reduced streak between spots in the pH area 7 to 9. It will 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.

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 to 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 to 7) and wide (3 to 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 to 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 with pH above 7.0 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.

Rehydration solution

DeStreak Reagent is compatible with most rehydration solutions when reducing agents like DTT or mercaptoethanol are removed.

Dissolve 15 mg (=12 µL) of DeStreak Reagent/mL of rehydration solution (without reducing agents).

Rehydrate and run

Rehydrate and run Immobiline DryStrip according to the short instructions attached to the Immobiline DryStrip package, or Handbook 2-D Electrophoresis: Principles and Methods Cytiva (Code no. 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

Storage:	4°C to 8°C.
Shelf life:	See expiry date on package.

Ordering information

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

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