

DeStreak Rehydration Solution

for IEF and 2-D electrophoresis

- Improves reproducibility of 2-D gels
 - prevents streaking
 - prevents non-specific oxidation
 - stabilizes protein patterns
- suitable for analytical and preparative gels

Introduction

The appearance of streaks that distort 2-D electrophoresis maps is a common problem, occurring most frequently when running gels that contain regions above pH 7.0. Increased sample load, increased length of the IPG strip or using a narrower pH gradient worsen the problem.

The appearance of extra spots, caused by non-specific oxidation of proteins, is another difficulty encountered when running gels containing basic pH regions. The number and intensity of these extra spots varies in a non-uniform manner between experiments. Extra spots can even appear in good quality gels generated with broad range IPG strips.

Streaking and non-specific oxidation result in poorly resolved protein patterns which are difficult to reproduce. Gels may need to be rerun and more spots need to be analyzed and identified, wasting time and resources.

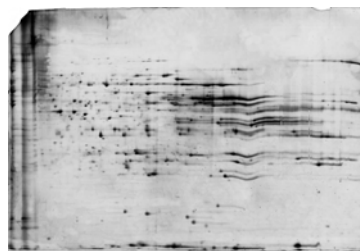
Using DeStreak™ Rehydration Solution in a 2-D gel protocol prevents non-specific oxidation and streaking, significantly improving reproducibility. Protein spots are well resolved and protein patterns are stabilized.

Prevents streaking

DeStreak Rehydration Solution stabilizes the protein components throughout an entire run. Stabilized

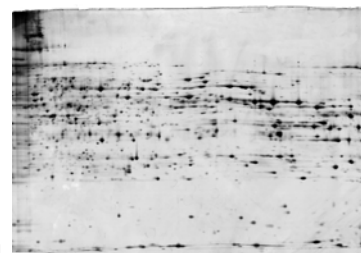


DeStreak Reagent and DeStreak Rehydration Solution.



Mouse liver proteins, 80 µg, Immobiline™ DryStrip, pH 6–9, 24 cm, rehydrated in 1% IPG Buffer pH 6–11, 8 M urea, 0.5% CHAPS and 10 mM DTT.

Mouse liver proteins, 80 µg, Immobiline DryStrip, pH 6–9, 24 cm rehydrated in DeStreak Rehydration Solution with 1% IPG Buffer pH 6–11.



components result in a simplified, well resolved pattern. When compared to the same sample run under reduced conditions using DTT (Figure 1), spots may move further towards the basic region of the gel (Figure 2).

Simplifies protein patterns by preventing non-specific oxidation

Samples analyzed under reduced conditions, using DTT with a run time of 10.6 kVh (Figure 3) or 25.3 kVh (Figure 4), show a typical pattern of increasing spot distribution over time. This phenomenon is caused by non-specific oxidation of proteins. The same analyses performed in the presence of DeStreak Rehydration Solution maintain stable, identical protein patterns even after longer focusing times (Figures 5 and 6).

Stabilizes patterns in IPG strips of any length and pH gradient

A comparison of results using 11 cm (Figure 5 and 6), 18 cm (Figure 7) or 24 cm (Figure 2) IPG strips in the first dimension shows the same stable, reproducible pattern in every analysis and for both of the basic pH range IPG strips pH interval 6–11 and 6–9.

Ensures well resolved patterns in preparative gels

The effectiveness of DeStreak Rehydration solution is maintained at the high sample loads and long run times required for preparative gel electrophoresis (Figure 8). There is no sign of non-specific oxidation after 63 kVh.

Identical samples run using a normal 2-D gel protocol with DTT Variable protein patterns

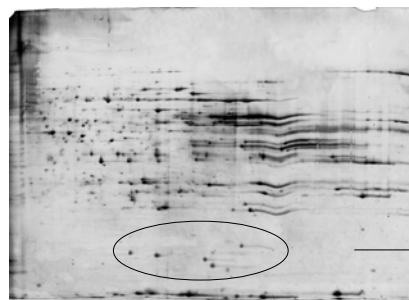


Fig 1. Mouse liver proteins, 80 µg, Immobiline DryStrip, pH 6–9, 24 cm, rehydrated in 1% IPG Buffer pH 6–11, 8 M urea, 0.5% CHAPS and 10 mM DTT.

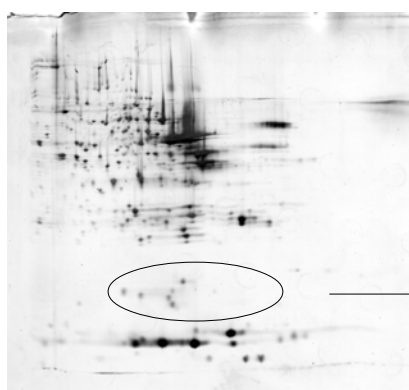


Fig 3. Mouse liver proteins, 20 µg, Immobiline DryStrip pH 6–11, 11cm, rehydrated in 1% IPG Buffer pH 6–11, 8 M urea, 0.5% CHAPS and 10 mM DTT. Run time 10.6 kVh.

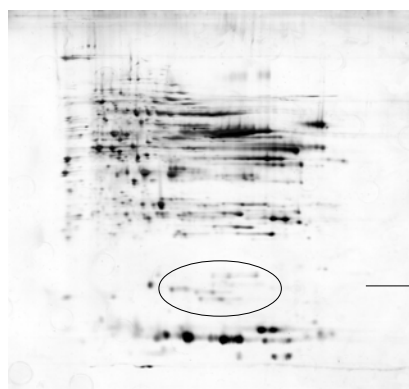


Fig 4. Mouse liver proteins, 20 µg, Immobiline DryStrip pH 6–11, 11cm, rehydrated in 1% IPG Buffer pH 6–11, 8 M urea, 0.5% CHAPS and 10 mM DTT. Run time 25.3 kVh.

Identical samples run using the same 2-D gel protocol with DeStreak Rehydration Solution

Stabilizes patterns in IPG strips of any length and pH gradient

Prevents streaking and non-specific oxidation

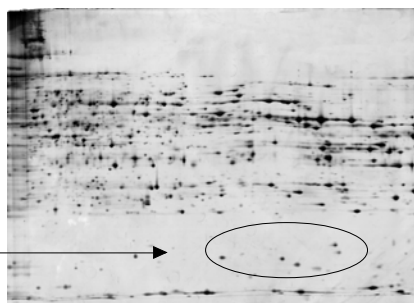


Fig 2. Mouse liver proteins, 80 µg, Immobiline DryStrip, pH 6–9, 24 cm, rehydrated in DeStreak Rehydration Solution with 1% IPG Buffer pH 6–11.

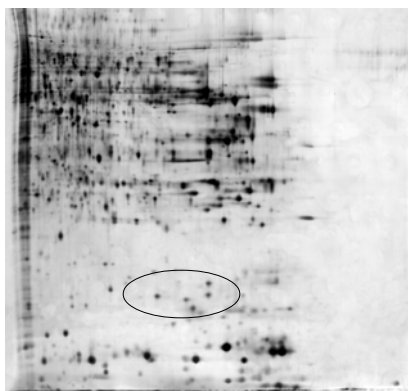


Fig 7. Mouse liver proteins, 96 µg, Immobiline DryStrip pH 6–11, 18 cm, rehydrated in 1% IPG Buffer pH 6–11 with DeStreak Rehydration Solution. Run 24 kVh.

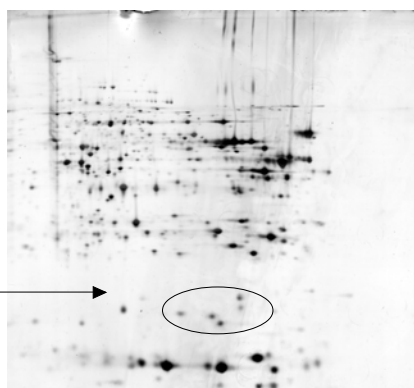


Fig 5. Mouse liver proteins, 20 µg, Immobiline DryStrip pH 6–11, 11cm, rehydrated in DeStreak Rehydration Solution with 1% IPG Buffer pH 6–11. Run time 10.6 kVh.

Well resolved patterns in preparatives gels

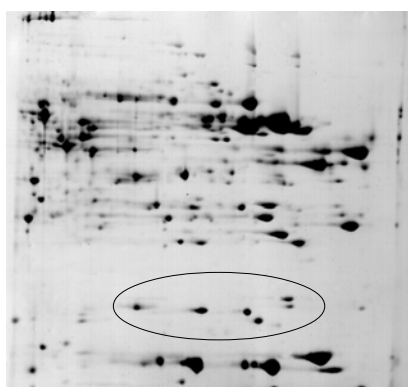


Fig 8. Mouse liver extract, 2 mg, Immobiline DryStrip, pH 6–9, 18 cm, rehydrated in 1% IPG Buffer pH 6–11 with DeStreak Rehydration Solution. Run 63 kVh.

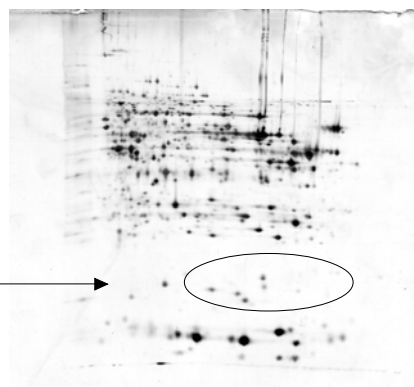


Fig 6. Mouse liver proteins, 20 µg, Immobiline DryStrip pH 6–11, 11cm, rehydrated in DeStreak Rehydration Solution with 1% IPG Buffer pH 6–11. Run time 25.3 kVh

Conclusion

Streaking and the appearance of extra spots can be linked to the oxidation state of thiol groups in the proteins under analysis. The number of extra spots varies with the number of oxidized thiols in each protein.

DeStreak Rehydration Solution maintains the protein thiol groups in one oxidation state, irrespective of sample load, pH range or run length. Using DeStreak Rehydration Solution in a 2-D gel protocol prevents streaking, simplifies the protein pattern by eliminating non-specific oxidation of proteins and produces highly reproducible, well resolved, stable spot patterns, free from distortion. DeStreak Rehydration Solution is as effective when used with high sample loads.

Sample conditions

Figs 1, 2, 3, 4, 5, 6. Sample in 8 M urea, 0.5% CHAPS, 1% Pharmalyte, pH 8–10.5 and 10 mM DTT.

Fig 7. Sample in 8 M urea, 2% CHAPS, 1% Pharmalyte, pH 8–10.5 and 10 mM DTT.

Fig 8. Sample in 8 M urea, 2% CHAPS, 1% Pharmalyte, pH 3–10 and 10 mM DTT, applied with paper bridge method.

Technical data

DeStreak Rehydration Solution contains optimized concentrations of urea, thiourea, CHAPS and DeStreak Reagent. The solution is ready for use after the addition of the appropriate IPG buffer.

DeStreak Reagent is also available.

Ordering information

Products	Quantity	Code Number
DeStreak Rehydration Solution	5 x 3 ml	17-6003-19
DeStreak Reagent	1 ml	17-6003-18

Related Products

Immobiline DryStrip, 7 cm

pH 4-7	12	17-6001-10
pH 3-10	12	17-6001-11
pH 3-10 NL	12	17-6001-12
pH 6-11	12	17-6001-94

Immobiline DryStrip, 11cm

pH 4-7	12	18-1016-60
pH 3-10	12	18-1016-61
pH6-11	12	17-6001-95

Immobiline DryStrip, 13cm

pH 4-7	12	17-6001-13
pH 3-10	12	17-6001-14
pH 3-10 NL	12	17-6001-15
pH6-11	12	17-6001-96

Immobiline DryStrip, 18cm

pH 4-7	12	17-1233-01
pH 3-10	12	17-1234-01
pH 3-10 NL	12	17-1235-01
pH 3.5-4.5	12	17-6001-83
pH 4.0-5.0	12	17-6001-84
pH 4.5-5.5	12	17-6001-85
pH 5.0-6.0	12	17-6001-86
pH 5.5-6.7	12	17-6001-87
pH 6-9	12	17-6001-88
pH 6-11	12	17-6001-97

Immobiline DryStrip, 24 cm

pH 3.5-4.5	12	17-6002-38
pH 4.0-5.0	12	17-6002-39
pH 4.5-5.5	12	17-6002-40
pH 5.0-6.0	12	17-6002-41
pH 5.5-6.7	12	17-6002-42
pH 3-7 NL	12	17-6002-43
pH 3-10	12	17-6002-44
pH 3-10 NL	12	17-6002-45
pH 4-7	12	17-6002-46
pH 6-9	12	17-6002-47

IPG buffers

IPG buffer pH 3.5-5.0	1 ml	17-6002-02
IPG buffer pH 4.5-5.5	1 ml	17-6002-04
IPG buffer pH 5.0-6.0	1 ml	17-6002-05
IPG buffer pH 5.5-6.7	1 ml	17-6002-06
IPG buffer pH 4-7	1 ml	17-6000-86
IPG buffer pH 6-11	1 ml	17-6001-78
IPG buffer pH 3-10 NL	1 ml	17-6000-88
IPG buffer pH 3-10	1 ml	17-6000-87

Chemicals

Pharmalyte™ 3-10	1 ml	17-0456-01
Urea	500 g	17-1319-01
CHAPS	1g	17-1314-01
Dithiothreitol (DTT)	1 g	17-1318-01
Bromophenol Blue	10 g	17-1329-01
Tris™	500 g	17-1321-01
Immobiline DryStrip Cover Fluid	1000 ml	17-1335-01

to order:

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