

Immobiline™ DryStrip

Instructions for Use

Technical data

Immobiline™ DryStrip gels contain pH gradient immobilized in homogeneous poly-acrylamide gels. The gels are cast on a plastic backing and delivered dried. Prior to use, they are rehydrated with a matching rehydration solution.

Package contents:	12 Immobiline DryStrip gels, and instructions	
Gel size:	69 × 3 × 0.5 mm	(7 cm)
	108 × 3 × 0.5 mm	(11 cm)
	128 × 3 × 0.5 mm	(13 cm)
	178 × 3 × 0.5 mm	(18 cm)
	235 × 3 × 0.5 mm	(24 cm)
Storage:	-20 °C	
Shelf life:	See expiry date on package	
Orientation:	The anode is + labeled on all strips except 11 cm 6-11, 13 cm 6-11 and 18 cm 6-11, which have their cathodes - labeled	

Sample and sample loading

Analytical sample loads

Use 2% pH 3-10 IPG buffer in the sample for all pH intervals. With the basic Immobiline DryStrip with pH intervals 6-9 and 6-11, we recommend anodic cup application. With the other pH intervals all sample application methods can be used, but sample-specific limitations may exist. When using the cup application, a maximum sample concentration of 100 µg protein/100 µL sample is recommended. Higher protein concentrations will lead to an increased risk of protein precipitation in the sample cup. A maximum of 150 µL sample solution may be applied to the sample cup. For plasma and other samples rich in serumalbumin, we recommend cathodic cup application and to use DTT in both sample and rehydration solution.

Suitable sample loads for protein stains and labels for the various pH gradients are shown in Table 2. Recommended suitable sample loads are based on results using total protein from *E. coli* extracts run on precast DALT Gel 12.5, ExcelGel™ gel, or on 1.0–1.5-mm thick vertical second-dimension gels. Using 2-D Quant Kit to determine the protein concentration prior to first-dimension IEF.

Preparative sample loads

See the Coomassie™ column in Table 2, for suitable preparative sample loads.

- For broad pH intervals (3–10, 3–10 NL, and 3–11 NL), preparative sample loads are preferably loaded in the rehydration step (rehydration loading).
- For neutral pH intervals (4-7) the sample is loaded in the rehydration step or by anodic paper-bridge loading.

- For basic pH intervals (6-9, and 6-11) preparative sample loads are applied using anodic paper-bridge loading. For 7 cm Immobiline DryStrip with basic pH interval, sample cup loading can be used as an alternative.

Using paper-bridge loading

The paper bridge soaked in sample is placed between the anode electrode and the Immobiline DryStrip (Fig 1).

Table 1. Maximum sample volume in paper-bridge loading

Immobiline DryStrip Length (cm)	7	11	13	18	24
Sample volume (µL)	150	300	400	400	400



Fig 1. Preparative sample application using paper-bridge loading

Minimize contaminants

When using large volumes of sample, the concentrations of salt, buffering substances, and other impurities that can interfere with the separation should be kept to a minimum. Such substances may prolong the required focusing time and may even disturb the pH gradient. For reducing sample volume and exchange buffer, Vivaspin™ columns or the 2D Clean-Up Kit may be an option. To get more information on how to remove impurities from your sample, see *2-D Electrophoresis: Principles and Methods*, see cytiva.com/handbook.

Table 2. Suitable sample loads for protein stains and labels

Immobiline DryStrip		Suitable sample load (µg of protein)		
		Silver	Coomassie (preparative)	CyDye™
7 cm	3–11 NL	3–6	25–60	10
7 cm	3–10	3–6	25–60	10
7 cm	3–10 NL	3–6	25–60	10
7 cm	4–7	4–8	25–150	13
7 cm	6–11	8–16	40–240	26
11 cm	3–11 NL	7–15	50–120	20
11 cm	3–10	7–15	50–120	20
11 cm	4–7	10–20	50–300	28
11 cm	6–11	20–40	100–600	56

Immobiline DryStrip		Suitable sample load (μg of protein)		
		Silver	Coomassie (preparative)	CyDye™
13 cm	3-11 NL	10-20	50-240	25
13 cm	3-10	10-20	50-240	25
13 cm	3-10 NL	10-20	50-240	25
13 cm	4-7	15-30	75-450	38
13 cm	6-11	30-60	150-900	76
18 cm	3-11 NL	20-40	100-500	50
18 cm	3-10	20-40	100-500	50
18 cm	3-10 NL	20-40	100-500	50
18 cm	4-7	30-60	150-900	75
18 cm	6-11	60-120	300-1500	150
24 cm	3-11 NL	30-60	200-600	100
24 cm	3-10	30-60	200-600	100
24 cm	3-10 NL	30-60	200-600	100
24 cm	4-7	45-90	200-1300	150
24 cm	6-9	80-200	400-2000	300

Rehydration

Note: Always wear laboratory gloves when handling Immobiline DryStrip gels and all apparatus/solutions used in their preparation to prevent contamination from skin keratin.

Step Action

- 1 Select an IPG Buffer with the same pH interval as the Immobiline DryStrip being rehydrated.

Note:

For Immobiline DryStrip pH interval	Use IPG Buffer pH Interval
6-9	6-11

- 2 Add the IPG Buffer to DeStreak Rehydration Solution or, if DTT is used, prepare an appropriate rehydration solution or lysis/sample solution. Use either DeStreak Reagent or DTT, not both.

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 3-11 NL is used. This will give high voltage and a short run time in hours, which is essential for streak free results.

Use 2 % IPG Buffer in the Rehydration Solution when:

- Higher protein solubility is needed.
- The sample contains a high salt concentration.

Note: Use of 2% IPG Buffer will give a higher conductivity and the highest voltage may not be reached.

Immobiline DryStrip gels are rehydrated individually in the IPGbox, the Immobiline DryStrip Reswelling Tray or the IPGphor Regular Strip Holder.

Sample can be applied by in-gel rehydration (included in rehydration solution) or by cup loading. When rehydration solution contains DeStreak, use cuploading only.

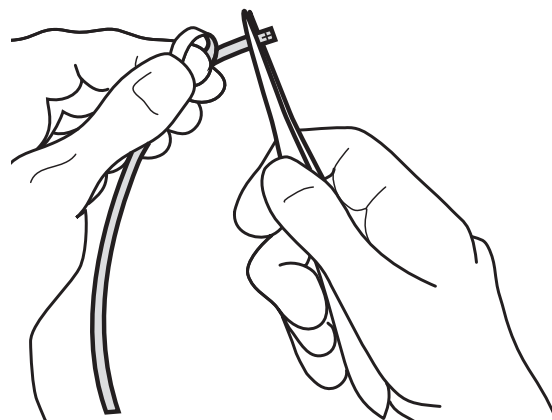
Step Action

- 1 Pipette the rehydration solution into the device chosen for rehydration. For volume, see Table 3. Distribute the solution evenly over the same length as the Immobiline DryStrip (7 cm to 24 cm).

Table 3. Rehydration solution volume

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

- 2 Carefully remove the cover foil from the Immobiline DryStrip. Start from the anode (+end).



Note:

Air bubbles under cover foil are normal.

- 3 Carefully place the Immobiline DryStrip in the reswelling tray channel with the gel side down. Take care to distribute the rehydration solution evenly under the strip. Avoid trapping air bubbles under the strip.
- 4 If IPGbox is used, close the lid. If Immobiline reswelling tray is used overlay the strip with Immobiline DryStrip Cover Fluid. Rehydrate for 10 to 20 h.

Guidelines and running conditions

General

Protocols and guidelines can be found on the following pages (Tables 4 to 13). If an overnight run is preferred, follow the instructions in the tables.

The protocols shown in tables with even numbers are suitable for firstdimension isoelectric focusing of proteins run on the Ettan™ IPGphor 3 Isoelectric Focusing Unit.

The protocols shown in tables with odd numbers are suitable for running Immobiline DryStrip gels on the Multiphor™ II Electrophoresis System connected to EPS 3501 XL Power Supply.

Note: The focusing times given are guidelines for well-prepared samples. Using crude samples with high protein and salt content or paper-bridge loading, or when there is a risk of precipitation of the sample, step 1 may be extended by up to 4 h to allow salt to migrate out of the strip at low voltages.

Ettan IPGphor 3 Isoelectric Focusing Unit

Soak the electrode pads in 150 µL distilled water and place them on top of the strip ends overlapping about half of the pads. For IPG strips exceeding pH 9, soak the cathodic electrode pad in 150 µL DeStreak rehydration solution instead of water.

- Using IPGphor Regular Strip Holder or Cup Loading Strip Holder the maximum allowed voltage is 8000 volts. Follow steps 1, 2, 3a and 4a when using 18 or 24 cm DryStrips.
- Using the IPGphor Cup Loading Manifold 10000 volts is allowed. Follow steps 1, 2, 3b and 4b when using 18 or 24 cm DryStrips.
- Running conditions: Temperature 20°C; current 50 µA per strip.

Same conditions for Ettan IPGphor II Isoelectric Focusing Unit as for Ettan IPGphor 3 Isoelectric Focusing Unit.

Multiphor II Electrophoresis System

Soak the 11 cm electrode pads in 0.5 mL distilled water and place them over the ends of the strips. For IPG strips exceeding pH 9, soak the cathodic electrode in 0.5 mL DeStreak rehydration solution instead of water.

Running conditions: Temperature 20°C; current 2 mA total; power 5 W total. Program EPS 3501 XL Power Supply in gradient mode and with **current check** option turned off.

7 cm Immobiline DryStrip

Table 4. Guidelines for running 7 cm Immobiline DryStrip gels on Ettan IPGphor 3 Isoelectric Focusing Unit.

pH intervals	Step	Voltage mode	Voltage (V)	Time (h:min)	kVh
3–11 NL	1	Step and Hold	300	0:30	0.2
3–10	2	Gradient	1000	0:30	0.3
6–11	3	Gradient	5000	1:20	0.4
	4	Step and Hold	5000	0:06-0:25	0.5-2.0
	Total			2:26-2:45	5.0-6.5
3–10 NL	1	Step and Hold	300	0:30	0.2
4–7	2	Gradient	1000	0:30	0.3
	3	Gradient	5000	1:30	4.5
	4	Step and Hold	5000	0:12-0:36	1.0-3.0
	Total			2:42-3:06	6.0-8.0

Table 5. Guidelines for running 7 cm Immobiline DryStrip gels on Multiphor II Electrophoresis System. Electrophoresis power supply in gradient mode for all steps.

pH intervals	Step	Voltage (V)	Time (h:min)	kVh
3–11 NL	1	200	0:01	
3–10	2	3500	1:30	2.8
6–11	3	3500	0:40-1:05	2.2-3.7
	Total		2:10-2:35	5.0-6.5
3–10 NL	1	200	0:01	
4–7	2	3500	1:30	2.8
	3	3500	0:55-1:30	3.2-5.2
	Total		2:25-3:00	6.0-8.0

11 cm Immobiline DryStrip

Table 6. Guidelines for running 11 cm Immobiline DryStrip gels on Ettan IPGphor 3 Isoelectric Focusing Unit.

pH intervals	Step	Voltage mode	Voltage (V)	Time (h:min)	kVh
3–11 NL	1	Step and Hold	500	1:00	0.5
3–10	2	Gradient	1000	1:00	0.8
6–11	3	Gradient	6000	2:00	7.0
	4	Step and Hold	6000	0:10-0:40	0.7-3.7
	Total			4:10-4:40	9.0-12.0
4–7	1	Step and Hold	500	1:00	0.5
	2	Gradient	1000	1:00	0.8
	3	Gradient	6000	2:30	8.8
	4	Step and Hold	6000	0:10-0:50	0.9-4.9
	Total			4:40-5:20	11.0-15.0

Table 7. Guidelines for running 11 cm Immobiline DryStrip gels on Multiphor II Electrophoresis System. Electrophoresis power supply in gradient mode for all steps.

pH intervals	Step	Voltage (V)	Time (h:min)	kVh
3–11 NL	1	300	0:01	
3–10	2	3500	1:30	2.9
6–11	3	3500	1:45-2:35	6.1-9.1
	Total		3:15-4:05	9.0-12.0
4–7	1	300	0:01	
	2	3500	1:30	2.9
	3	3500	2:20-3:30	8.1-12.1
	Total		3:50-5:00	11.0-15.0

13 cm Immobiline DryStrip

Table 8. Guidelines for running 13 cm Immobiline DryStrip gels on Ettan IPGphor 3 Isoelectric Focusing Unit.

pH intervals	Step	Voltage mode	Voltage (V)	Time (h:min)	kVh
3–11 NL	1	Step and Hold	500	1:00	0.5
3–10	2	Gradient	1000	1:00	0.8
6–11	3	Gradient	8000	2:30	11.3
	4	Step and Hold	8000	0:10-0:30	1.4-4.4
	Total			4:40-5:00	14.0-17.0
3–10 NL	1	Step and Hold	500	1:00	0.5
4–7	2	Gradient	1000	1:00	0.8
	3	Gradient	8000	2:30	11.3
	4	Step and Hold	8000	0:25-0:55	3.4-7.4
	Total			4:55-5:25	16.0-20.0

Table 9. Guidelines for running 13 cm Immobiline DryStrip gels on Multiphor II Electrophoresis System. Electrophoresis power supply in gradient mode for all steps.

pH intervals	Step	Voltage (V)	Time (h:min)	kVh
3–11 NL	1	300	0:01	
3–10	2	3500	1:30	2.9
6–11	3	3500	3:10-4:00	11.1-14.1
	Total		4:40-5:30	14.0-17.0
3–10 NL	1	300	0:01	
4–7	2	3500	1:30	2.9
	3	3500	3:45-5:10	13.1-18.1
	Total		5:15-6:40	16.0-21.0

18 cm Immobiline DryStrip

Table 10. Guidelines for running 18 cm Immobiline DryStrip gels on Ettan IPGphor 3 Isoelectric Focusing Unit.

pH intervals	Step	Voltage mode	Voltage (V)	Time (h:min)	kVh
3-11 NL	1	Step and Hold ¹	500	1:00 (8:00) ¹	0.5
3-10	2	Gradient	1000	1:00	0.8
6-11	3a	Gradient ²	8000	3:00	13.5
	4a	Step and hold ²	8000	4:46-1:30	6.2-12.2
	3b	Gradient ³	10000	3:00	16.5
	4b	Step and Hold ³	10000	0:20-0:55	3.2-9.2
	Total				21.0-27.0
3-10 NL	1	Step and Hold ¹	500	1:00 (8:00) ¹	0.5
4-7	2	Gradient	1000	1:00	0.8
	3a	Gradient ²	8000	3:00	13.5
	4a	Step and Hold ²	8000	1:30-2:40	12.2-21.2
	3b	Gradient ³	10000	3:00	16.5
	4b	Step and Hold ³	10000	0:55-1:50	9.2-18.2
	Total				27.0-36.0

¹ When a more convenient overnight run of 15 to 17 h is desired, the time in step 1 can be extended up to recommended value in brackets. Using this option, step 4 can be reduced with the added kVh in step 1, to reach the specified total kVh.

² Follow steps 1, 2, 3a and 4a when using IPGphor Regular Strip Holder or Cup Loading Strip Holder.

³ Follow steps 1, 2, 3b and 4b when using IPGphor Cup Loading Manifold.

Table 11. Guidelines for running 18 cm Immobiline DryStrip gels on Multiphor II Electrophoresis System. Electrophoresis power supply in gradient mode for all steps.

pH intervals	Step	Voltage (V)	Time (h:min)	kVh
3-11 NL	1	500	0:01	
3-10	2	3500	1:30	3.0
6-11	3	3500	4:50-6:20	17.0-22.0
	Total		6:20-7:50	20.0-25.0
3-10 NL	1	500	0:01	
4-7	2 ¹	500	6:00	3.0
	3	3500	1:30	3.0
	4	3500	5:25-9:25	19.0-30.0
	Total		12:55-16:55	25.0-36.0

¹ This step is added to give a convenient overnight run (15 h). This step may be omitted. Step 4 should then be extended by 2.5 kVh.

24 cm Immobiline DryStrip

Table 12. Guidelines for running 24 cm Immobiline DryStrip gels on Ettan IPGphor 3 Isoelectric Focusing Unit.

pH intervals	Step	Voltage mode	Voltage (V)	Time (h:min)	kVh
3-11 NL	1	Step and Hold ¹	500	1:00 (8:00) ¹	0.5
3-10	2	Gradient	1000	1:00	0.8
	3a	Gradient ²	8000	3:00	13.5
	4a	Step and Hold ²	8000	2:30-3:45	20.0-30.0
	3b	Gradient ³	10000	3:00	16.5
	4b	Step and Hold ³	10000	1:45-2:45	17.2-27.2
	Total				35.0-45.0
3-10 NL	1	Step and Hold ¹	500	1:00 (7:00) ¹	0.5

pH intervals	Step	Voltage mode	Voltage (V)	Time (h:min)	kVh
4-7	2	Gradient	1000	1:00	0.8
	3a	Gradient ²	8000	3:00	13.5
	4a	Step and Hold ²	8000	3:45-5:36	30.0-45.0
	3b	Gradient ³	10000	3:00	16.5
	4b	Step and Hold ³	10000	2:45-4:15	27.2-42.2
	Total				45.0-60.0
6-9	1	Step and Hold ¹	500	1:00 (5:00) ¹	0.5
	2	Gradient	1000	1:00	0.8
	3a	Gradient ²	8000	3:00	13.5
	4a	Step and Hold ²	8000	5:36-8:45	45.0-70.0
	3b	Gradient ³	10000	3:00	16.5
	4b	Step and Hold ³	10000	4:15-6:45	42.2-67.2
	Total				60.0-85.0

¹ When a more convenient overnight run of 15 to 17 h is desired, the time in step 1 can be prolonged to up to recommended value in brackets. Using this option, step 4 can be reduced with the added kVh in step 1, to reach the specified total kVh.

² Follow steps 1, 2, 3a and 4a when using IPGphor Regular Strip Holder or Cup Loading Strip Holder.

³ Follow steps 1, 2, 3b and 4b when using IPGphor Cup Loading Manifold.

Table 13. Guidelines for running 24 cm Immobiline DryStrip gels on Multiphor II Electrophoresis System. Electrophoresis power supply in gradient mode for all steps.

pH intervals	Step	Voltage (V)	Time (h:min)	kVh
3-11 NL	1	500	0:01	
3-10	2 ¹	500 ¹	5:00 ¹	2.5
	3	3500	1:30	3.0
	4	3500	8:30-11:20	29.5-39.5
	Total		15:00-17:50	35.0-45.0
3-10 NL	1	500	0:01	
4-7	2	3500	1:30	3.0
	3	3500	12:00-16:20	42.0-57.0
	Total		13:30-17:50	45.0-60.0
6-9	1	500	0:01	
	2	3500	1:30	3.0
	3	3500	16:20-22:00	57.0-77.0
	Total		17:50-23:30	60.0-80.0

¹ This step is added to give a convenient overnight run (15 h). This step can be omitted. Step 4 should then be extended by 2.5 kVh.

Ordering information

Immobiline DryStrip	Quantity	Code Number
7 cm, pH 3–11 NL	12	17-6003-73
7 cm, pH 4–7	12	17-6001-10
7 cm, pH 3–10	12	17-6001-11
7 cm, pH 3–10 NL	12	17-6001-12
7 cm, pH 6–11	12	17-6001-94
11 cm, pH 3–11 NL	12	17-6003-74
11 cm, pH 4–7	12	18-1016-60
11 cm, pH 3–10	12	18-1016-61
11 cm, pH 6–11	12	17-6001-95
13 cm, pH 3–11 NL	12	17-6003-75
13 cm, pH 4–7	12	17-6001-13
13 cm, pH 3–10	12	17-6001-14
13 cm, pH 3–10 NL	12	17-6001-15
13 cm, pH 6–11	12	17-6001-96
18 cm, pH 3–11 NL	12	17-6003-76
18 cm, pH 4–7	12	17-1233-01
18 cm, pH 3–10	12	17-1234-01
18 cm, pH 3–10 NL	12	17-1235-01
18 cm, pH 6–11	12	17-6001-97
24 cm, pH 3–11 NL	12	17-6003-77
24 cm, pH 3–10	12	17-6002-44
24 cm, pH 3–10 NL	12	17-6002-45
24 cm, pH 4–7	12	17-6002-46
24 cm, pH 6–9	12	17-6002-47

IPG Buffer	Quantity	Code Number
pH 4–7	1 mL	17-6000-86
pH 3–10	1 mL	17-6000-87
pH 3–10 NL	1 mL	17-6000-88
pH 3–11 NL	1 mL	17-6004-40
pH 6–11	1 mL	17-6001-78

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