



# Immobiline DryStrip

Rehydration and running conditions for Immobiline DryStrip gels

## Technical data

Immobiline™ DryStrip gels contain a preformed 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 acidic pH intervals (3.5–4.5 and 3.5–6 NL), we recommend rehydration loading or cathodic sample cup application. With neutral narrow range pH intervals (3-7 NL, 4-7 and 5.3-6.5), all sample application methods can be used, but sample-specific limitations may exist. With basic Immobiline DryStrip with pH extending above 7 (pH intervals 6.2–7.5, 6–9, 6–11, 3–10, 3–10 NL, 3–11 NL and 7–11 NL), we recommend anodic cup application. 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 a 1.0–1.5-mm thick vertical second-dimension gels. Use 2-D Quant Kit to determine the protein concentration prior to first-dimension IEF.

## Preparative sample loads

See Table 2, Coomassie, for suitable preparative sample loads.

- For broad pH intervals (3–10, 3–10 NL, and 3–11 NL) and acidic pH intervals (3.5–6 NL and 3.5–4.5), preparative sample loads are preferably loaded in the rehydration step (rehydration loading).
- For neutral pH intervals (3-7 NL, 4-7 and 5.3-6.5) the sample is loaded in the rehydration step or by anodic paper-bridge loading.
- For basic pH intervals (6.2–7.5, 6–9, 6–11, and 7–11 NL), preparative sample loads are applied using anodic paper-bridge loading. For 7 cm Immobiline Drystrip with basic pH interval, sample cup loading is 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 disturbing impurities from your sample, see 2-D Electrophoresis: Principles and Methods, Sample preparation.

**Table 2.** Suitable sample loads for protein stains and labels

Immobiline DryStrip	Suitable sample load (µg of protein)		
	Silver /Deep Purple™	Coomassie (preparative)	CyDye
7cm 3-11 NL	3-6	25-60	10
7cm 3-10	3-6	25-60	10
7cm 3-10 NL	3-6	25-60	10
7cm 4-7	4-8	25-150	13
7cm 3-5.6 NL	8-16	40-240	26
7cm 5.3-6.5	8-16	40-240	26
7cm 6.2-7.5	8-16	40-240	26
7cm 6-11	8-16	40-240	26
7cm 7-11NL	8-16	40-240	26
11cm 3-11 NL	7-15	50-120	20
11cm 3-10	7-15	50-120	20
11cm 4-7	10-20	50-300	28
11cm 3-5.6 NL	20-40	100-600	56
11cm 5.3-6.5	20-40	100-600	56
11cm 6.2-7.5	20-40	100-600	56
11cm 6-11	20-40	100-600	56
11cm 7-11NL	20-40	100-600	56
13cm 3-11 NL	10-20	50-240	25
13cm 3-10	10-20	50-240	25
13cm 3-10 NL	10-20	50-240	25
13cm 4-7	15-30	75-450	38
13cm 3-5.6 NL	30-60	150-900	76
13cm 5.3-6.5	30-60	150-900	76
13cm 6.2-7.5	30-60	150-900	76
13cm 6-11	30-60	150-900	76
13cm 7-11NL	30-60	150-900	76

### Suitable sample load (µg of protein)

Immobiline DryStrip	Silver	Coomassie	CyDye
	/Deep Purple	(preparative)	
18cm 3-11 NL	20-40	100-500	50
18cm 3-10	20-40	100-500	50
18cm 3-10 NL	20-40	100-500	50
18cm 4-7	30-60	150-900	75
18cm 3-5.6 NL	60-120	300-1500	150
18cm 5.3-6.5	60-120	300-1500	150
18cm 6.2-7.5	60-120	300-1500	150
18cm 6-9	60-120	300-1500	150
18cm 6-11	60-120	300-1500	150
18cm 7-11NL	60-120	300-1500	150
24cm 3-11 NL	30-60	200-600	100
24cm 3-10	30-60	200-600	100
24cm 3-10 NL	30-60	200-600	100
24cm 4-7	45-90	200-1300	150
24cm 3-7 NL	45-90	200-1300	150
24cm 3-5.6 NL	80-200	400-2000	300
24cm 3.5-4.5	80-200	400-2000	300
24cm 5.3-6.5	80-200	400-2000	300
24cm 6.2-7.5	80-200	400-2000	300
24cm 6-9	80-200	400-2000	300
24cm 7-11NL	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.

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

<b>Note:</b> For Immobiline DryStrip	Use IPG Buffer
pH interval	pH interval
3.5-4.5, 3-5.6 NL	3.5-5.0
5.3-6.5	5.5-6.7
6-9, 6.2-7.5	6-11

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 7-11 NL and 3-11 NL are 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:

- 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.

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 cup-loading only.

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 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/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 on how to prolong the time.

The protocols shown in tables with **even** numbers are suitable for first-dimension 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 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 cm or 24 cm Drystrips.
- Using the IPGphor Cup Loading Manifold 10000 volts is allowed. Follow steps 1, 2, 3b and 4b when using 18 cm or 24 cm Drystrips.

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.

### 7 cm

**Table 4.** Guidelines for running 7 cm Immobiline DryStrip gels on **Ettan IPGphor 3 Isoelectric Focusing Unit**. Running conditions: Temperature 20 °C; current 50 µA per strip

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	4.0
	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
3-5.6 NL	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
7-11 NL	1 Step and Hold	300	0:30	0.2
	2 Gradient	1000	1:00	0.7
	3 Gradient	5000	1:30	4.5
	4 Step and Hold	5000	0:20-0:55	1.6-4.6
	Total		3:20-3:55	7.0-10.0
5.3-6.5	1 Step and Hold	300	1:00	0.2
6.2-7.5	2 Gradient	1000	1:00	0.7
	3 Gradient	5000	2:30	7.5
	4 Step and Hold	5000	0:45-1:30	3.6-7.6
	Total		5:15-6:00	12.0-16.0

**Table 5.** Guidelines for running 7 cm Immobiline DryStrip gels on **Multiphor II Electrophoresis System**. 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.

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
3-5.6 NL	3	3500	0:55-1:30	3.2-5.2
	Total		2:25-3:00	6.0-8.0
7-11 NL	1	300	0:01	
	2	3500	1:30	2.9
	3	3500	1:10-2:02	4.1-7.1
	Total		2:40-3:30	7.0-10.0
5.3-6.5	1	300	0:01	
6.2-7.5	2	3500	1:30	2.9
	3	3500	2:36-3:45	9.1-13.1
	Total		4:06-5:15	12.0-16.0

# 11 cm

**Table 6.** Guidelines for running 11 cm Immobiline DryStrip gels on **Ettan IPGphor 3 Isoelectric Focusing Unit**. Running conditions: Temperature 20 °C; current 50 µA per strip

pH intervals	Step Voltage mode	Voltage (V)	Time (h:min)	kVh
3-11 NL	1 Step and Hold	500	1:00	0.5
	2 Gradient	1000	1:00	0.8
3-10	3 Gradient	6000	2:00	7.0
	4 Step and Hold	6000	0:10-0:40	0.7-3.7
6-11	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
3-5.6 NL	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
7-11 NL	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	0:50-1:40	4.9-9.9
	Total		5:20-6:10	15.0-20.0
5.3-6.5	1 Step and Hold*	500	1:00*	0.5
	2 Gradient	1000	1:00	0.8
6.2-7.5	3 Gradient	6000	3:00	10.5
	4 Step and Hold	6000	2:40-3:50	16.2-23.2
	Total		7:40-8:50	28.0-35.0

\* To convert this to a convenient overnight run, extend Step 1 to 6 h (3 kVh) and reduce step 4 with 3 kVh.

**Table 7.** Guidelines for running 11 cm Immobiline DryStrip gels on **Multiphor II Electrophoresis System**. 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.

pH intervals	Step	Voltage (V)	Time (h:min)	kVh
3-11 NL	1	300	0:01	
	2	3500	1:30	2.9
3-10	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
3-5.6 NL	3	3500	2:20-3:30	8.1-12.1
	Total		3:50-5:00	11.0-15.0
7-11 NL	1	300	0:01	
	2	3500	1:30	2.9
	3	3500	3:30-4:55	12.1-17.1
	Total		5:00-6:25	15.0-20.0
5.3-6.5	1*	500*	0:01*	
	2	3500	1:30	3.0
6.2-7.5	3	3500	7:10-9:10	25.0-32.0
	Total		8:40-10:40*	28.0-35.0

\* To adjust this protocol to an overnight run, extend step 1 by 5 h (2.5 kVh) and reduce step 3 by 2.5 kVh.

# 13 cm

**Table 8.** Guidelines for running 13 cm Immobiline DryStrip gels on **Ettan IPGphor 3 Isoelectric Focusing Unit**. Running conditions: Temperature 20 °C; current 50 µA per strip

pH intervals	Step Voltage mode	Voltage (V)	Time (h:min)	kVh
3-10	1 Step and Hold	500	1:00	0.5
3-11 NL	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
3-5.6 NL	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
7-11 NL	1 Step and Hold	500	1:00	0.5
	2 Gradient	1000	1:00	0.8
	3 Gradient	8000	3:00	13.5
	4 Step and Hold	8000	0:45-1:15	6.2-10.2
	Total		5:45-6:15	21.0-25.0
5.3-6.5	1 Step and Hold*	500	1:00*	0.5
6.2-7.5	2 Gradient	1000	1:00	0.8
	3 Gradient	8000	3:00	13.5
	4 Step and Hold	8000	2:55-4:10	23.2-33.2
	Total		7:55-9:10	38.0-48.0

\* To convert this to a convenient overnight run, extend step 1 to 6 h (3 kVh) and reduce step 4 with 3 kVh.

**Table 9.** Guidelines for running 13 cm Immobiline DryStrip gels on **Multiphor II Electrophoresis System**. 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.

pH intervals	Step	Voltage (V)	Time (h:min)	kVh
3-10	1	300	0:01	
3-11 NL	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
3-5.6 NL	3	3500	3:45-5:10	13.1-18.1
	Total		5:15-6:40	16.0-21.0
7-11 NL	1	500	0:01	
	2	3500	1:30	3.0
	3	3500	5:10-6:20	18.1-22.0
	Total		6:40-7:50	21.0-25.0
5.3-6.5	1*	500*	0:01*	
6.2-7.5	2	3500	1:30	3.0
	3	3500	10:00-12:50	35.0-45.0
	Total		11:30-14:20	38.0-48.0

\* To adjust this protocol to an overnight run, extend the time of step 1 to 2h.

# 18 cm

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

pH interval	Step Voltage mode	Voltage (V)	Time (h:min)	kVh
3-10	1 Step and Hold *	500	1:00 (8:00)*	0.5
3-11 NL	2 Gradient	1000	1:00	0.8
6-11	3a Gradient †	8000	3:00	13.5
	4a Step and Hold †	8000	0:46-1:30	6.2-12.2
	3b Gradient †	10000	3:00	16.5
	4b Step and Hold †	10000	0:20-0:55	3.2-9.2
	Total			21.0-27.0
3-10NL	1 Step and Hold*	500	1:00 (8:00)*	0.5
4-7	2 Gradient	1000	1:00	0.8
3-5.6 NL	3a Gradient †	8000	3:00	13.5
	4a Step and Hold †	8000	1:30-2:40	12.2-21.2
	3b Gradient †	10000	3:00	16.5
	4b Step and Hold †	10000	0:55-1:50	9.2-18.2
	Total			27.0-36.0
6-9	1 Step and Hold*	500	1:00 (8:00)*	0.5
7-11 NL	2 Gradient	1000	1:00	0.8
	3a Gradient †	8000	3:00	13.5
	4a Step and Hold †	8000	3:10-4:30	25.2-35.2
	3b Gradient †	10000	3:00	16.5
	4b Step and Hold †	10000	2:15-3:15	22.2-32.2
Total			40.0-50.0	
5.3-6.5	1 Step and Hold*	500	2:00 (3:00)*	1.0
6.2-7.5	2 Gradient	1000	2:00	1.5
	3a Gradient †	8000	3:00	13.5
	4a Step and Hold †	8000	6:45-8:40	54.0-69.0
	3b Gradient †	10000	3:00	16.5
	4b Step and Hold †	10000	5:05-6:35	51.0-66.0
Total			70.0-85.0	

\* 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.

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

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

**Table 11.** Guidelines for running 18 cm Immobiline DryStrip on **Multiphor II Electrophoresis System**. 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.

pH interval	Step	Voltage (V)	Time (h:min)	kVh
3-10	1	500	0:01	
3-11 NL	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
4-7	1	500	0:01	
3-10 NL	2*	500	6:00	3.0
3-5.6 NL	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
6-9	1	500	0:01	
7-11 NL	2*	500	3:00	1.5
	3	3500	1:30	3.0
	4	3500	10:10-13:00	35.5-45.5
	Total		14:40-17:30	40.0-50.0
5.3-6.5	1	500	0:01	
6.2-7.5	2	3500	1:30	3.0
	3	3500	19:10-23:25	67.0-82.0
	Total		20:40-24:55	70.0-85.0

\* 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

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

pH interval	Step	Voltage mode	Voltage (V)	Time (h:min)	kVh
3-11 NL 3-10	1	Step and Hold*	500	1:00 (8:00)*	0.5
	2	Gradient	1000	1:00	0.8
	3a	Gradient †	8000	3:00	13.5
	4a	Step and Hold †	8000	2:30-3:45	20.0-30.0
	3b	Gradient †	10000	3:00	16.5
	4b	Step and Hold †	10000	1:45-2:45	17.2-27.2
Total					35.0-45.0
3-10 NL 3-7 NL 4-7 3-5.6 NL	1	Step and Hold*	500	1:00 (7:00)*	0.5
	2	Gradient	1000	1:00	0.8
	3a	Gradient †	8000	3:00	13.5
	4a	Step and Hold †	8000	3:45-5:36	30.0-45.0
	3b	Gradient †	10000	3:00	16.5
	4b	Step and Hold †	10000	2:45-4:15	27.2-42.2
Total					45.0-60.0
6-9 7-11 NL	1	Step and Hold*	500	1:00 (5:00)*	0.5
	2	Gradient	1000	1:00	0.8
	3a	Gradient †	8000	3:00	13.5
	4a	Step and Hold †	8000	5:36-8:45	45.0-70.0
	3b	Gradient †	10000	3:00	16.5
	4b	Step and Hold †	10000	4:15-6:45	42.2-67.2
Total					60.0-85.0
3.5-4.5	1	Step and Hold	500	2:00 (5:00)*	1.0
	2	Gradient	1000	2:00	1.5
	3a	Gradient †	8000	3:00	13.5
	4a	Step and Hold †	8000	9:10-10:30	74.0-84.0
	3b	Gradient †	10000	3:00	16.5
	4b	Step and Hold †	10000	7:05-8:05	71.0-81.0
Total					90.0-100.0

**(Table 12 continued)**

5.3-6.5	1 Step and Hold	500	2:00 (5:00)*	1.0	
6.2-7.5	2 Gradient	1000	2:00	1.5	
	3a Gradient	8000	3:00	13.5	
	4a Step and Hold	8000	11:45-14:15	94.0-114.0	
	3b Gradient	10000	3:00	16.5	
	4b Step and Hold	10000	9:05-11:05	91.0-111.0	
Total					110.0-130.0

\* 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.

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

‡ 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.** 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.

pH intervals	Step	Voltage (V)	Time (h:min)	kVh
3-11 NL 3-10	1	500	0:01	
	2*	500*	5:00*	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 3-7 NL 4-7 3-5.6 NL	1	500	0:01	
	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 7-11 NL	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
3.5-4.5	1	500	0:01	
	2	3500	1:30	3.0
	3	3500	22:00-27:40	77.0-97.0
	Total		23:30-29:10	80.0-100.0



**(Table 13 continued)**

5.3-6.5	1	500	0:01	
6.2-7.5	2	3500	1:30	3.0
	3	3500	30:35-36:20	107.0-127.0
	Total		32:06-37:50	110.0-130.0

\* 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 3-5.6 NL	12	17-6003-53
7 cm, pH 5.3-6.5	12	17-6003-58
7 cm, pH 6.2-7.5	12	17-6003-63
7 cm, pH 7-11 NL	12	17-6003-68
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 3-5.6 NL	12	17-6003-54
11 cm, pH 5.3-6.5	12	17-6003-59
11 cm, pH 6.2-7.5	12	17-6003-64
11 cm, pH 7-11 NL	12	17-6003-69
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 3-5.6 NL	12	17-6003-55
13 cm, pH 5.3-6.5	12	17-6003-60
13 cm, pH 6.2-7.5	12	17-6003-65
13 cm, pH 7-11 NL	12	17-6003-70
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

Immobiline Drystrip	Quantity	Code Number
18 cm, pH 3-5.6 NL	12	17-6003-56
18 cm, pH 5.3-6.5	12	17-6003-61
18 cm, pH 6.2-7.5	12	17-6003-66
18 cm, pH 7-11 NL	12	17-6003-71
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 6-9	12	17-6001-88
18 cm, pH 6-11	12	17-6001-97
24 cm, pH 3-5.6 NL	12	17-6003-57
24 cm, pH 3.5-4.5	12	17-6002-38
24 cm, pH 5.3-6.5	12	17-6003-62
24 cm, pH 6.2-7.5	12	17-6003-67
24 cm, pH 7-11 NL	12	17-6003-72
24 cm, pH 3-11 NL	12	17-6003-77
24 cm, pH 3-7 NL	12	17-6002-43
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 3.5-5.0	1 ml	17-6002-02
pH 5.5-6.7	1 ml	17-6002-06
pH 7-11 NL	1 ml	17-6004-39
pH 6-11	1 ml	17-6001-78

## Related products

Urea	500 g	17-1319-01
CHAPS	1 g	17-1314-01
Triton X-100	500 ml	17-1315-01
DeStreak Reagent	1ml	17-6003-18
DeStreak Rehydration solution	5 x 3 ml	17-6003-19
Immobiline DryStrip Cover Fluid	1000 ml	17-1335-01
2-D Protein Extraction Buffer Trial Kit	For 6x10ml	28-9435-22
2-D Protein Extraction Buffer-I	For 50 ml	
(Not compatible with CyDye DIGE minimal dyes)		28-9435-23
2-D Protein Extraction Buffer-II	For 50 ml	28-9435-24
2-D Protein Extraction Buffer-III	For 50 ml	
(Not compatible with CyDye DIGE saturation dyes)		28-9435-25
2-D Protein Extraction Buffer-IV	For 50 ml	
(Not compatible with CyDye DIGE saturation dyes)		28-9435-26
2-D Protein Extraction Buffer-V	For 50 ml	28-9435-27
2-D Protein Extraction Buffer-VI	For 50 ml	28-9435-28
CyDye DIGE Fluor Minimal dye labeling kit	2 nmol	28-9345-30
Dithiothreitol	1 g	17-1318-01
Bromophenol Blue	10 g	17-1329-01
Coomassie PhastGel R-350	40 tablets	17-0518-01
Deep Purple Total Protein Stain	25 ml	RPN6306
Thiourea	100 g	RPN6301
Electrode set		80-6498-76
Sample cups	120	80-6498-95
IPGbox		28-9334-65
IPGbox kit		28-9334-92
2-D Quant Kit		80-6483-56
2-D Clean-Up Kit		80-6484-51
Vivaspin 2, (MWCO 3000)	25	28-9322-40
Multiphor II Electrophoresis Unit		18-1018-06
EPS 3501 XL Power Supply		18-1130-05
Ettan IPGphor 3 Isoelectric Focusing Unit		11-0033-64
Ettan IPGphor Manifold, complete		80-6498-38
IPGphor Strip Holders		Inquire

## Related literature

2-D Electrophoresis: Principles and Methods	80-6429-60
Ettan DIGE System User manual	18-1173-17

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