PhastSystem, PhastGel media, and accessories

Multi-tasking workstation for electrophoretic analysis of proteins, peptides, and nucleic acids

Introduction

PhastSystem™ is a multi-tasking, semi-automated electrophoresis workstation that runs up to two gels, under the same conditions within a separation and control unit, while staining up to two gels in a development unit.

- Automated workstation for separation, blotting, and staining (Fig 1)
- Reproducible separations using precast PhastGel™ media and reagents for SDS- and native-PAGE, IEF, 2-D PAGE and DNA separations
- Publication-quality results with minimal effort

  Separation: < 60 min
  Protein blotting: < 30 min
  Detection of protein or DNA: < 60 min

Reproducible separations

A wide range of precast PhastGel™ separation media ensures optimal results for isoelectric focusing (IEF), native or SDS-PAGE, 2-D electrophoresis and DNA separations. Ready-to-use PhastGel Buffer Strips for protein or DNA separations, eliminate the need for buffer preparation.

Rapid detection of proteins, peptides and DNA

PhastSystem offers complete flexibility in the choice of detection method. Ready-to-use reagents and optimized development protocols save time and improve reproducibility. To ensure reliable results, programming instructions are supplied for automated Coomassie™ staining with PhastGel BlueR and silver staining with PhastGel Protein or PhastGel DNA Silver Staining Kits. Other detection methods such as PAS glycoprotein staining, autoradiography or enzyme activity assays can also be used. If required, proteins and peptides can be transferred to immobilization membranes by semi-dry electrophoretic transfer using PhastTransfer Kit, prior to detection.

Ready in minutes for publication

After development, gels are left to air dry or dried quickly (5–10 min) using a hairdryer or microwave oven. PhastGel media do not crack or distort so results are easily stored, ready for publication.

Fig 1. Multi-tasking PhastSystem

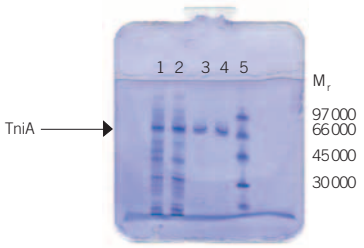
Separations by native or SDS-PAGE

Proteins, peptides or DNA are separated according to size on homogenous or gradient gels. The appropriate gel is selected according to the molecular size range in the sample and the degree of resolution required between the components.

Gradient gels are best suited for running unknown samples, giving tight bands, but a compressed pattern. Broad continuous gradients (8–25%) are suitable for separating a wide molecular size range whereas 4–15% is designed for separation of high molecular weight proteins, and 10–15% is designed for the separation of medium molecular weight proteins.

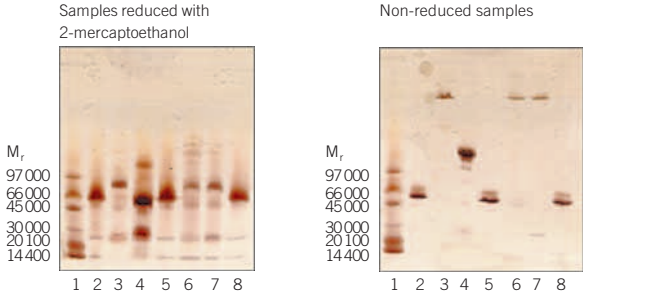
Homogenous gels (7.5%, 12.5% and 20%) provide high resolution for a narrow size range. PhastGel Homogenous High Density has been specifically optimized for the separation of smaller molecules such as peptides and oligonucleotides.

The same type of gel can be used for native or SDS-PAGE separations. The nature of the separation is determined by selection of the appropriate buffer strips: PhastGel PAGE Buffer Strips, PhastGel SDS-PAGE Buffer Strips or PhastGel DNA Buffer Strips. These ready-to-use, agarose buffer strips contain the buffer required for each separation. Figure 2 shows examples of protein, peptide and DNA separations on a range of PhastGel media. Recommended separation ranges for each gel are given under Technical specifications in this datafile.



Lane 1. Sample, clarified extract diluted 5-fold
Lane 2. Pooled from SOURCE™ 15Q 4.6/100 PE
Lane 3. Pooled from HiTrap™ Heparin
Lane 4. Pooled from Mono S 5/50 GL
Lane 5. LMW-SDS Marker Kit

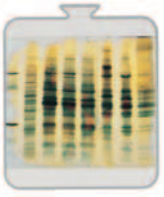
Analysis of fractions from a chromatographic purification of a DNA binding protein, transposase TniA. SDS-PAGE, PhastGel Homogenous 12.5 Coomassie stained



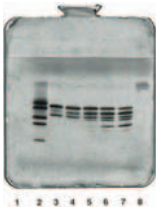
Lane 1. Low Molecular Weight Calibration Kit
Lane 2. Cell culture supernatant, starting material, diluted 20-fold
Lane 3. IgM, human
Lane 4. IgG
Lane 5. Flow-through pool, diluted 20-fold
Lane 6. Eluted IgM, fraction 8, diluted 8-fold
Lane 7. Eluted IgM, fraction 9, diluted 8-fold
Lane 8. Washing out unbound material, pool diluted 3-fold

Reduced and non-reduced fractions from purification of monoclonal a-Shigella IgM from hybridoma cell culture supernatant. Analysis demonstrated a purity level of over 80%. SDS-PAGE, PhastGel Gradient 4–15

Detection: PhastGel Protein Silver Staining Kit, typical sensitivity 1 to 5 ng protein/band native-PAGE.



Comparison of Mycoplasma strains. SDS-PAGE, PhastGel Gradient 10–15 Proteins transferred to nitrocellulose membrane, using PhastTransfer.



Separation of peptides PhastGel Homogenous High Density

Analysis of crude PCR reaction products on PhastGel Homogeneous 12.5, silver stained.

Fig 2. Examples of native and SDS-PAGE separations on homogenous or gradient PhastGel media using a range of detection methods.

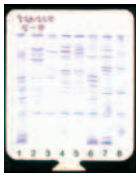
Isoelectric focusing

For separations according to differences in isoelectric point (pI), PhastGel IEF media provide stable, linear pH gradients (3–9, 4–6.5 and 5–8) with uniform conductivity across broad and narrow pH ranges. PhastGel IEF media withstand field strengths >500 V/cm for high resolution separations. PhastGel Dry IEF is supplied precast and dried. This gel contains no residual acrylamide monomers or other contaminants from the polymerization process, thereby offering greater versatility and reliable band separation. Before use, the gel is reswollen with the chosen carrier ampholyte solution and other additives as required for the application. Reswelling takes place within a PhastGel Cassette to ensure controlled, reproducible rehydration. The process takes from 0.5–2 h depending on the composition of the rehydration solution. Guidance for choosing rehydration solutions is supplied with the product. Examples of IEF separations are shown in Figure 3.
A dedicated PhastGel kit is available for the detection of oligoclonal IgG in unconcentrated cerebrospinal fluid, the CSF Analysis Kit includes gels and reagents for 40 analyses.

Development processes for detection of bands are similar to native and SDS-PAGE gels.

IEF separation of *E.coli* proteins.
PhastGel Dry IEF, Pharmalyte™ carrier ampholytes 2.5–5 and 5–8, urea, Triton X-100.

Detection: PhastGel Protein Silver Staining Kit, typical sensitivity 1 to 5 ng protein/band native-PAGE.



Gadoid and other fish proteins separated on PhastGel IEF 5–8 followed by Coomassie staining. Lane 1, Hake (*Merluccius merluccius*); lane 2, Whiting (*Merlangius merlangus*); lane 3,Kingklip (*Genypterus blacodes*); lane 4, Cod (*Gadus morhua*); lane 5, Alaska Pollock (*Theragra chalcogramma*); lane 6, Saithe (*Pollachius virens*); lane 7, Haddock (*Melanogrammus aeglefinus*); lane 8, Cusk (*Brosme brosme*).

Analysis of phosphoglucomutase activity in meat proteins
PhastGel IEF 5–8

Detection: enzyme activity assay

Fig 3. Examples of IEF separations using alternative detection methods.

Simple, controlled separations

Programming

Up to nine separation methods and nine development methods can be programmed directly and stored within the PhastSystem separation and control unit. Help messages appear on screen to assist with programming or warn of programming errors. Methods can be named, edited and programmed while the system is running. A battery back-up ensures that no methods are lost in the event of a power failure and a diagnostic check, performed each time the unit is switched on, warns of any technical problems. The actual separation or staining conditions can be monitored in real time throughout the experiment.

Separation methods

Each separation method contains instructions to lower and raise the sample applicators, an extra alarm instruction and up to nine other steps that are used to specify run conditions at the programmed time. For each step the following parameters are entered:

- Voltage: 1 to 2000 V
- Current: 0.1 to 50 mA
- Power: 0.1 to 7.0 W
- Separation temperature: 0 –70 °C
- Volthours: 1 to 9999 Vh/step

Development methods

Each development method can contain up to 20 steps, allowing full flexibility for optimization of staining and destaining protocols. For each step the following parameters are entered:

- IN-port: the port through which solutions enter the development unit (ports 1 to 9 can be used, port 0 is reserved for waste
- OUT-port: the port through which solutions exit
- Duration of the step in minutes (maximum 99.9 min per step)
- Processing temperature for the step (maximum 50 °C). Note that the development chamber can only warm up solutions from ambient temperature.

Figure 4 illustrates the step-by-step simplicity of separation and detection when using PhastSystem. Times refer to a typical DNA separation followed by silver staining.

Position the gel, buffer strips and electrodes 2 min

Load your sample for automatic application 2 min

Run the separation 25 min

Transfer the gel to the development chamber … 1min

… for automated silver staining 45 min

Fig 4. Separation and detection on PhastSystem, step-by-step.

Easy set-up of gels and buffers

PhastGel media are precast and ready to use eliminating time-consuming and often unreliable gel casting. Agarose-based PhastGel Buffer Strips eliminate buffer preparation steps. The electrode solutions for native, SDS-PAGE or DNA separations are contained in the strips which are placed on

both ends of the gel. The electrodes rest on the surface of the strips. No electrode solutions are required for IEF and the electrodes rest directly on the gel surface.

Accurate sample loading and application

A sample applicator is selected according to the number of samples and the volume that is to be loaded on the gel. The comb-like applicators are available in 12 × 0.3 µl, 8 × 0.5 µl, 8 × 1 µl and 6 × 4 µl formats. Samples are loaded simply by dipping the comb into sample droplets held in place on a sample-well stamp (Fig 5). The capillary wells of the PhastGel sample applicators hold samples until the applicator is lowered onto the gel after the correct, preprogrammed period of pre-electrophoresis. The applicators are then raised to avoid the risk of band disturbances.

Fig 5. Applicator comb draws up identical sample volumes from droplets on a sample-well stamp.

Ensuring high resolution and reproducibility

Up to two gels can be run simultaneously under the same conditions within the separation unit as illustrated in Figure 6. To ensure high resolution and high reproducibility, conditions during the electrophoretic separation are precisely controlled.

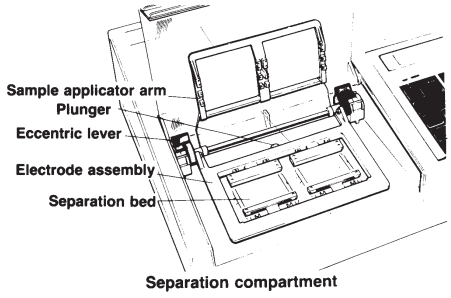


Fig 6. Separation compartment in the separation and control unit.

Accurate temperature control

The high field strengths required for fast, high resolution separation can generate significant increases in temperature, but constant temperature is essential for reproducible results. The programmable temperature range extends from 0 –70 °C. However, the cooling capacity of the separation bed depends on the ambient temperature, humidity, and the power applied to the gels. The temperature of the separation bed is therefore measured at one second intervals and fed back to an in-built Peltier element that cools or warms the bed, regulating the running temperature. For standard runs, the bed can be cooled to approximately 15 °C below ambient temperature and held to within ±1 °C.

Accurate power supply

The power supply in the separation and control unit operates in one of three modes: constant current, constant voltage or constant power. Limits on these parameters are set when programming the method. PhastSystem automatically adjusts the parameters during a run. The power supply accurately delivers voltage and current even at low field strengths (10 V, 0.1 mA). Up to 540 V/cm can be applied to the gels, ideal for high resolution IEF.

Volthour control

For maximum reproducibility, PhastSystem uses volthours, to regulate the duration of separation and the period for sample application. A volthour integrator continuously integrates the voltage applied with respect to time controlling protein migration in the gel.

Fast, reproducible detection

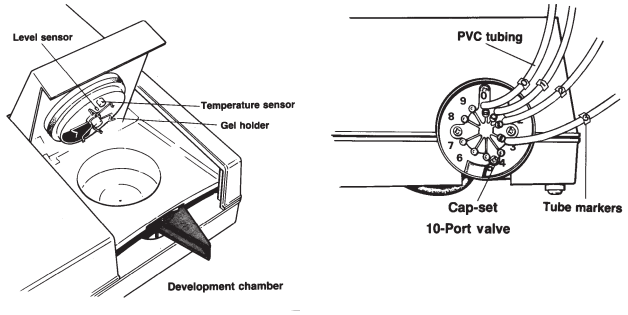


Fig 7. PhastSystem development unit.

After separation, gels can be transferred directly to the development unit where the combination of thin PhastGel media, elevated temperature and controlled rotation under optimized conditions ensures rapid development.

Staining and destaining take place in the stainless steel development chamber (Fig 7) under controlled temperatures and for preprogrammed time periods. A gel holder, liquid level sensor and temperature sensor are mounted in the lid of the chamber. Solutions are pumped into and out of the chamber through one of 10 ports by a pneumatic pump, flowing to and from the ports via PVC tubing. When solution enters the chamber, it is heated to the programmed temperature for that step. The time required to attain the required temperature depends on the initial temperature. Typically solutions can be heated from 20 –50 °C within 3–4 min. The temperature is held constant to within ±2 °C for the remainder of the step. The gel holder subjects the gels to pulsed rotation within the solution.

After the specified time period, the pump forces solution out of the chamber to be recycled or sent to waste. Hazardous chemicals can be collected separately. The chamber is then filled with the next solution according to the programmed method and the process is repeated. Approximately 80 ml of liquid is required for each fill.

Blotting in practice

PhastTransfer provides a fast, reproducible solution for semi-dry electrophoretic transfer of proteins from PhastGel media to immobilization membranes. The small format of PhastTransfer minimizes the requirements for buffers and detection reagents. Components are shown in Figure 8. The gel backing remover efficiently removes the plastic backing from PhastGel media. The electrodes are designed to ensure uniform contact with the transfer 'sandwich'. The electrodes prevent air bubbles forming at the gel surface and rapidly dissipate the heat generated. The PhastTransfer Kit fits into the separation compartment of PhastSystem and run parameters are programmed as for a separation. Up to two gels can be processed in a single run, with a recommended power of 1.0 mA/cm^2. For most proteins, transfer efficiency greater than 90% is achievable within 10–30 min. Figure 9 illustrates the simplicity of the blotting process. After transfer, proteins can be visualized using general or specific detection methods. The membranes can be processed automatically within the PhastSystem development unit if the detection method is appropriate.

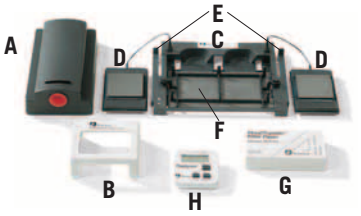


Fig 8. Components of PhastTransfer Kit
A = mounting block, **B** = cutting frame with stainless steel wire, **C** = base plate with anode electrodes, **D** = cathode electrodes, **E** = contact leads, **F** = transfer frame, **G** =filter paper, **H** = timer

Load transfer cassette

Place membrane on the gel, followed by wetted filter papers

Remove gel backing

Place wetted filter papers onto anode plate, followed by gel, membrane and filter papers

Place cathode electrode in position and lower lid

Run the transfer

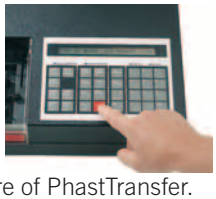


Fig 9. Simple blotting procedure of PhastTransfer.

Technical specifications

PhastSystem

Operation

Safety certification	CE 89/336/EEC (EMC directive), CE 73/23/EEC (LV directive), EN-61010-1 (IEC1010-1, UL3101-1, CSA22.2 1010-1
Room temp.	4–40 °C
Humidity	Maximum 95%
Mains voltage	100/120 VAC or 220/240 VAC ±10%
Mains frequency	48–62 Hz
Power consumption	Maximum 174 W/unit

Separation and control unit

Capacity	1 or 2 gels
Programmable parameters	
Voltage	10–2000 V, error ≤ 3% of actual value (≤ 5 V for 10–200 V)
Current	0.1–50.0 mA, error ≤ 2% of actual value (≤ 0.2 mA for 0.1–5.0 mA)
Power	0.1–7.0 W, error ≤ 6% of actual value (≤ 0.3 W for 0.1–1.0W)
Temperature	0–70 °C
Volthours	1–9999 volthours/step
Sample application	12 × 0.3, 8 × 0.5, 8 × 1.0 and 6 × 4 µl
Alarm	Alarm sounds at end of separation methods and at an optimal step

Safety features

Power supply switched off and alarm sounds if separation lid is opened during run.

Memory backup	Lithium battery
DC Keyboard	31 touch type keys with audible tone
Display	40 digit alphanumeric liquid crystal
LEDs	4 LEDs for status information
Cooling/heating	Peltier element
Electrode material	platinized titanium (1 cathode and 2 anodes/gel)
Dimensions	460 × 300 × 138 mm (W × L × H)
Weight	6.2 kg

Development unit

Development	Up to 9 solutions are automatically pumped into and out of the chamber or to waste. Gels rotate during development.
Capacity	1 or 2 gels
Volume	80 ml solution required
Programmable parameters	
Temperature	Ambient up to 50 °C ±2 °C (≤ 4 min, to heat from 20 to 50 °C)
Ports	9, plus 1 for waste.
Time	maximum 99.9 min per step
Alarm	Alarm sounds at the end of the development process
Materials	Chamber, gel holder, temp. sensor in stainless steel Level sensor enclosed in glass
Chemical resistance	Compatible with solutions used for Coomassie and silver staining. Avoid ketones, strong acids and organic solvents.
Dimensions	300 × 300 × 138 mm (w × l × h)
Weight	4.8 kg

PhastTransfer

Capacity	1 or 2 gels
Transfer time	10–30 min
Buffer volume	5 ml/gel
Power supply	PhastSystem Separation and Control Unit
Programmable parameters	
Voltage	1–2000 V
Current	0.1–50 mA
Power	1–7 W
Temperature	1–70 °C
Volthours	1–9999 Vh/step
Heating /cooling	Peltier elements
Electrodes	One pair for each gel, made of PBTP (thermoplastic polyester containing glass fiber) and graphite
Chemical resistance	Aqueous solvents and diluted alcohols, pH 2–11. Avoid use of acetone, phenol, halogenated hydrocarbons and undiluted alcohols
Dimensions	
Transfer unit	200 mm × 150 mm × 30 mm (w × d × h), 0.74 kg
Gel backing remover	170 mm × 90 mm × 60 mm (w × d × h), 0.24 kg

PhastGel media and buffer strips

Buffer Strips

Dimensions (W × L × H)	10 × 41 × 6 mm
Material	3% agarose
Buffer content:	
PhastGel PAGE Buffer Strips	0.88 M L-alanine, 0.25 M Tris, pH 8.8
PhastGel SDS-PAGE Buffer Strips	0.20 M tricine, 0.20 M Tris, 0.55% SDS, pH 8.1
PhastGel DNA Buffer Strips*	0.20 M tricine, 0.20 M Tris, 0.55% SDS, pH 8.1
Storage	4–8 °C, maximum 12 months

* Each batch of PhastSystem DNA Buffer Strips undergoes a functionality test to ensure suitability for DNA separation.

PhastGel media

Materials	Polyacrylamide on GelBond film backing + buffer or carrier ampholytes

Native and SDS-PAGE	PhastGel Gradient			PhastGel Homogenous			PhastGel Homogenous High Density
% acrylamide	4–15	10–15	8–25	7.5	12.5	20	20 (optimized for low M_r separations)
Separation range: native ($M_r \times 10^3$)	70–700	80–440	50–300	230–700	80–230	50–100	20–60
Separation range: DS ($M_r \times 10^3$)	30–200	15–150	6–150	40–200	20–45	3–25	2–15
DNA separation range base pair	180–2000	100–1500	50–1000	280–1500	100–400	35–200	20–50
Stacking zone (13 mm) composition (% T/% C)	4.5/3	6/3	6/3	5/3	6/3	7.5/2	7.5/2
Separating zone (32 mm) composition (% T/% C)	5–15/2	10–15/2	8–25/2	7.5/2	12.5/2	20/2	20/2
Buffer	0.112 M acetate, 0.112 M Tris, pH 6.4						0.112 M acetate, 0.112 M Tris, pH 6.4, 30% ethylene glycol
Storage	4–8 °C, maximum 9 months						
Dimensions (W × L × T)	43 × 50 × 0.45 mm						

Isoelectric focusing	PhastGel IEF			PhastGel Dry IEF
Separation range (pI)	3–9	4–6.5	5–8	Optional
Composition (%T/%C)	5/3	5/3	5/3	5/3
Buffers	Pharmalyte™ carrier ampholytes			Reswell in chosen carrier ampholyte solution
Storage	4–8 °C, maximum 18 months			–20–80 °C, maximum 18 months
Dimensions (W × L × T)	43 × 50 × 0.45 mm			

Staining reagents

PhastGel Blue R

Coomassie R 350 dye in tablet format
One tablet makes 400 ml, 0.1% staining solution
Typical sensitivity: 50–100 ng protein/ band
Time to result: 25–45 min, according to gel type.

PhastGel Protein Silver Staining Kit

Reagents and development protocol optimized for PhastGel media.
Typical sensitivity: 1–5 ng protein/band for IEF and native-PAGE
0.3–0.5 ng protein/band for SDS-PAGE.
Time to result: 60–75 min, according to gel type.

PhastGel DNA Silver Staining Kit

Reagents and development protocols optimized for PhastGel media.
Typical sensitivity: 20–50 pg nucleic acid/band.
Time to result: 30 min

Ordering information

	Quantity	Code no.
PhastSystem Separation-Control and Development Units 120 V		18-1018-23
PhastSystem Separation-Control and Development Units 220 V		18-1018-24
Separation-Control Unit 120 V		18-1200-00
Separation-Control Unit 220 V		18-1200-10
Related products		
PhastGel media		
PhastGel Gradient 10–15	10	17-0540-01
PhastGel Gradient 8–25	10	17-0542-01
PhastGel Gradient 4–15	10	17-0678-01
PhastGel Homogeneous 7.5	10	17-0622-01
PhastGel Homogeneous 12.5	10	17-0623-01
PhastGel Homogeneous 20	10	17-0624-01
PhastGel Homogenous-High density	10	17-0679-01
PhastGel IEF 3–9	10	17-0543-01
PhastGel IEF 4–6.5	10	17-0544-01
PhastGel IEF 5–8	10	17-0545-01
PhastGel cassette (for IEF)	1	18-1001-01
PhastGel Dry IEF	10 pairs	17-0677-01
PhastGel Buffer Strips-SDS	10 pairs	17-0516-01
PhastGel Buffer Strips-Native	10 pairs	17-0517-01
PhastGel Buffer Strips-DNA	10 pairs	17-1599-01
PhastGel Staining Kits		
PlusOne™ Coomassie Tablets, PhastGel Blue R-350	40 tablets	17-0518-01
PhastGel Protein Silver Staining Kit	1	17-0617-01
PhastGel DNA Silver Staining Kit	1	17-1596-01
PhastGel Sample Applicators		
PhastGel Sample Applicator 12 × 0.3 µl	50	18-1614-01
PhastGel Sample Applicator 8 × 0.5 µl	50	18-1617-01
PhastGel Sample Applicator 8 × 1 µl	50	18-1618-01
PhastGel Sample Applicator 6 × 4 µl	50	18-0012-29
Sample-well stamp	1	18-0097-01

	Quantity	Code no.
PhastTransfer		
PhastTransfer Kit includes gel backing remover, electrode cassette and filter paper	1	18-1001-23
PhastTransfer Filter Paper (50 × 50 mm)	200/pk	18-1003-18
Nitrocellulose blotting membranes		
0.45 µm, 150 × 200 mm	15 sheets	25-8000-45
0.45 µm, 0.2 × 3.0 m	3 meters	25-8000-46
0.2 µm, 150 × 200 mm	15 sheets	25-8000-47
Protein molecular weight markers		
LMW-SDS Marker Kit	10 vials 575 µg/vial	17-0446-01
HWM-SDS Marker Kit	10 vials 175 µg/vial	17-0615-01
HMW-Native Marker Kit	10 vials 250 µg/vial	17-0445-01
Peptide Marker Kit	1 vial 2 mg/vial	80-1129-83
pI markers		
Broad pI Kit, pH 3–10	10 vials	17-0471-01
Low pI Kit, pH 2.5–6.5	10 vials	17-0472-01
High pI Kit, pH 5–10.5	10 vials	17-0473-01
Carbamylyte Calibration Kit	1	17-0582-01
DNA molecular weight markers		
ΦX-174 RF DNA *Hae* III Digest (72–1 353 bp)	10 µg	27-4044-01
Oligonucleotide Sizing Markers (8–32 bases)	25 reactions	27-2521-01
Application kit		
CSF Analysis Kit for detection of oligoclonal IgG in unconcentrated cerebrospinal fluid	1	18-1039-14

to order:

Asia Pacific Tel: +852 2811 8693 Fax: +852 2811 5251 **Australia** Tel: +61 2 9899 0999 Fax: +61 2 9899 7511 **Austria** Tel: 01 57 606 16 19 Fax 01 57 606 16 27 **Belgium** Tel: 0800 73 888 Fax: 03 272 1637 **Canada** Tel: 1 800 463 5800 Fax: 1 800 567 1008 **Central, East, South East Europe** Tel: +43 1 982 3826 Fax: +43 1 985 8327 **Denmark** Tel: 45 16 2400 Fax: 45 16 2424 **Finland & Baltics** Tel: +358 (0)9 512 3940 Fax: +358-(0)9-512 39439 **France** Tel: 0169 35 67 00 Fax: 0169 41 9677 **Germany** Tel: 0761 4903 490 Fax: 0761 4903 405 **Italy** Tel: 02 27322 1 Fax: 02 27302 212 **Japan** Tel: 81 3 5331 9336 Fax: 81 3 5331 9370 **Latin America** Tel: +55 11 3933 7300 Fax: +55 11 3933 7304 **Middle East** Tel: +30 2 10 96 00 687 Fax: +30 2 10 96 00 693 **Netherlands** Tel: 0165 580 410 Fax: 0165 580 401 **Norway** Tel: 815 65 555 Fax: 815 65 666 **Portugal** Tel: 21 417 7035 Fax: 21 417 3184 **Russia & other C.I.S. & N.I.S.** Tel: +7 (095) 232 0250,956 1137 Fax: +7 (095) 230 6377 **South East Asia** Tel: 60 3 8024 2080 Fax: 60 3 8024 2090 **Spain** Tel: 93 594 49 50 Fax: 93 594 49 55 **Sweden** Tel: 018 612 19 00 Fax: 018 612 19 10 **Switzerland** Tel: 0848 8028 12 Fax 0848 8028 13 **UK** Tel: 0800 616 928 Fax: 0800 616 927 **USA** Tel: +1 800 526 3593 Fax: +1 877 295 8102

Amersham Biosciences UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, United Kingdom. Amersham Biosciences AB, SE-751 84 Uppsala, Sweden. Amersham Biosciences Corp., 800 Centennial Avenue, PO Box 1327, Piscataway, NJ 08855, USA. Amersham Biosciences Europe GmbH, Munzinger Strasse 9, D-79111, Freiburg, Germany.

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