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# ProductInformation

**AutoPAGE™ PLUS 4.5%**
**Acrylamide solution for automated sequencing**

Product No. **P 8977**
Store at 2-8 °C

## Product Description

AutoPAGE Plus has been optimized for use with either 2X or 3X gel modules for ABI Prism™ 377 instruments. AutoPAGE Plus delivers improved resolution and longer read lengths with shorter run times.

## Precautions and Disclaimer

Acrylamide is a neurotoxin. Handle with care. For laboratory use only. Not for drug, household or other uses.

Reagents Required But Not Provided

- Ammonium persulfate (APS), Product No. A 9164
- TEMED, Product No. T 7024
- 1X Tris-Borate-EDTA (TBE) Buffer, prepared by diluting 10X TBE (Product No. T 4415) 10-fold with water
- ABI Prism™ 377 48 cm glass plates, Product No. Z37,793-7 or ABI Prism™ 377 36 cm glass plates, Product No. Z37,792-9

## Preparation Instructions

A. Gel preparation

To 50 ml of AutoPAGE solution add 35 µl of TEMED and 250 µl of 10% APS. Mix the solution by slowly swirling. The addition of APS and TEMED initiates polymerization. The gel should be poured as soon as possible, preferably within 2 minutes. Degassing and filtering the gel solution before casting is optional, but not necessary.

B. Gel Casting

Before casting the gel thoroughly clean front and back plates along with the 0.2 mm spacer. Make sure the inside of the plate is free of water droplets, dust, lint or anything else that might fluoresce or scatter light. To assemble the plates, plate the 0.2 mm gel spacers on the rear plate and align the front plate (inside down) on the spacer. In order to keep the spacers in position, apply water droplets to the edge of the bottom plate before setting the spacers in place. The bottom ends of the plates should be flush with each other and the notch of the front plate should be oriented toward the top of the gel. Cast the gel and insert comb according to standard procedures. Allow 1.5-2 hours for the gel to polymerize. Gels may be stored overnight at 4 °C. Before storing, cover both ends of the gel cassette with absorbent towels soaked in 1X TBE buffer and wrap gel with plastic wrap to avoid drying.

C. Preparing for Electrophoresis

Remove the comb slowly and clean the outside of the plates to remove any excess acrylamide or dirt. Prepare approximately 1.5 liters of 1X TBE electrophoresis buffer to fill both the upper and lower buffer chambers of the instrument. Mount the gel cassette onto the sequencing apparatus according to the manufacturer's instructions. Begin a plate check to determine the cleanliness of the plates. If the plates are not clean, remove them from the mount and clean them again. Repeat the above step as needed.

## Procedure

A. Instructions for running an ABI Prism 377 with 36 and 48 cm plates

Prepare an analysis matrix standard file for the AutoPAGE gel solution as described in the ABI Prism 377 automated sequencer user's manual. Prepare the sample sheet as normal. Pre-run the gel per standard methodology or per ABI user's manual. After the pre-run is complete, set instrument to pause and rinse the wells thoroughly with approximately 10 ml 1X TBE buffer. All wells intended for use should be thoroughly rinsed and free of residual gel particles. Load prepared samples and begin the instrument's run function, using the desired run module.

B. Optimized Electrophoresis Conditions

The optimized electrophoresis conditions for 48 cm plates require the default settings to be changed from 2.4 kV to 2.88 kV. The combination of these conditions and the AutoPAGE Plus formulation will consistently yield 700 well-resolved bases in 6.5-7.0 hours with up to 900 bases in 8.5 hours (see table under Results). AutoPAGE Plus can also be used with 36 cm plates with similar results and no modifications to the default conditions necessary. 48 cm plates run under default conditions (2.4 kV) using AutoPAGE Plus will yield 700 well-resolved bases in 8 hours.

**Results**

Typical laboratory results for AutoPAGE Plus with 48 cm plates are shown below. Similar results are observed with 36 cm plates.

| Plate size | KV | No. of Bases | Approx. Time (hr) |
|---|---|---|---|
| 48 cm | 2.88 | 700 | 6.5 |
| 48 cm | 2.88 | 800 | 7.5 |
| 48 cm | 2.88 | 900 | 8.5 |

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