

# flashPAGE™ Reaction Clean-Up Kit

Part Number AM12200



## A. Product Description

The flashPAGE™ Reaction Clean-Up Kit is a fast and convenient filter-based purification/concentration system for nucleic acids obtained with the flashPAGE Fractionator. During flashPAGE electrophoresis, RNA and DNA molecules smaller than 40 nt in length are separated from longer species and are collected in the lower running buffer. It is possible to precipitate the nucleic acids from the electrophoresis buffer using sodium acetate and ethanol, however, for quantitative recovery, the precipitation must be incubated overnight at  $-20^{\circ}\text{C}$  (find a protocol link at [www.ambion.com/catalog/CatNum.php?12200](http://www.ambion.com/catalog/CatNum.php?12200)). We developed the flashPAGE Reaction Clean-Up Kit as a rapid and simple alternative to overnight precipitation. Using the flashPAGE Fractionator and flashPAGE Reaction Clean-Up Kit, the entire small nucleic acid purification procedure takes less than 45 minutes, providing significant time and labor savings compared to traditional PAGE purification and overnight precipitation. Purified nucleic acids can be used for any downstream enzymatic or analytical applications, including array analyses, solution hybridization, and Northern blotting.

The flashPAGE Reaction Clean-Up Kit procedure consists of the following steps:

- Add flashPAGE Filter Binding Additive and ethanol to sample and mix thoroughly.
- Pass sample through a Filter Cartridge which retains the small nucleic acids.
- Wash the Filter Cartridge.
- Elute purified nucleic acid in  $\geq 30\ \mu\text{L}$  of Nuclease-free Water.

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## B. Kit Contents and Storage Conditions

The flashPAGE Reaction Clean-Up Kit contains reagents for purification of 20 samples obtained using the flashPAGE Fractionator.

Amount	Component	Storage
25 mL	flashPAGE Wash Solution Concentrate (Add 20 mL 100% ethanol before use)	4°C/room temp
1.75 mL	Nuclease-free Water	any temp*
10 mL	flashPAGE Filter Wetting Solution	room temp
2 x 1.5 mL	flashPAGE Filter Binding Additive	room temp
20	Filter Cartridges and Tubes	room temp
20	Elution Tubes	room temp

\* Nuclease-free Water can be stored at  $-20^{\circ}\text{C}$ ,  $4^{\circ}\text{C}$ , or room temp.

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## C. Required Materials Not Provided With the Kit

- 100% ethanol: ACS grade or better
- Incubators set to  $95^{\circ}\text{C}$  and  $65\text{--}70^{\circ}\text{C}$
- Microcentrifuge (required)
- Vacuum manifold (optional)

Using a vacuum manifold is considerably faster than drawing the solutions through the Filter Cartridges with a microcentrifuge. Use 3 mL syringe barrels to support the Filter Cartridges on the vacuum manifold.

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## D. Buffer and Equipment Preparation

### Add 20 mL 100% ethanol to the flashPAGE Wash Solution Concentrate and mix well

To prepare flashPAGE Wash Solution, add 20 mL of high quality 100% ethanol to the bottle labeled flashPAGE Wash Solution Concentrate. Mix thoroughly, and mark the label to indicate that the ethanol was added.

## RNase precautions

### Lab bench and pipettors

Before working with RNA, it is always a good idea to clean the lab bench and pipettors with an RNase decontamination solution (e.g. Ambion® RNaseZap® Solution).

### Gloves and RNase-free technique

Wear laboratory gloves at all times during this procedure and change them frequently. They protect you from the reagents, and they protect nucleic acids from nucleases that are present on skin.

Use RNase-free pipette tips for this procedure, and avoid putting used tips into the reagent containers.

## Preheat the Nuclease-free Water and a 65–70°C incubator

- Heat the Nuclease-free Water to 95°C; preheated Nuclease-free Water will be used to elute the purified nucleic acids in step 7.
- Elution (step 7) requires an incubation at 65–70°C. We typically incubate in a heat block or a hybridization oven, but any 65–70°C incubator can be used.

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## E. flashPAGE Reaction Clean-Up Kit Procedure



### CAUTION

*Do not centrifuge the Filter Cartridges at forces over RCF ~10,000 x g because it could cause mechanical damage and/or may deposit glass filter fiber in the final sample.*

### 1. Prewet a Filter Cartridge by drawing 500 µL flashPAGE Filter Wetting Solution through it.

- a. Pipet 500 µL flashPAGE Filter Wetting Solution into a Filter Cartridge/Tube to prewet the filter. Draw the solution through the filter by centrifuging the Filter Cartridge/Tube at 2,500 x g for about 1 min.
- b. Discard the flashPAGE Filter Wetting Solution from the tube and either return the Filter Cartridge to the tube, or position it on a 3 mL syringe barrel on a vacuum manifold.

## 2. Roughly measure the sample volume after flashPAGE fractionation.

After flashPAGE fractionation, small nucleic acids will be in the Lower Running Buffer; its volume should be 230  $\mu\text{L}$   $\pm$  10  $\mu\text{L}$ .

- If the volume of the Lower Running Buffer is below 220  $\mu\text{L}$ , bring the solution to 230  $\mu\text{L}$  with Nuclease-free Water.
- If the volume is more than about 240  $\mu\text{L}$ , it indicates that there may be a leak in the flashPAGE Gel which would allow Upper Buffer to bypass the gel and enter the lower buffer chamber. See flashPAGE troubleshooting suggestions by following the link at:  
[www.ambion.com/catalog/CatNum.php?12200](http://www.ambion.com/catalog/CatNum.php?12200)

## 3. Add 135 $\mu\text{L}$ flashPAGE Filter Binding Additive to the Lower Running Buffer and mix well.

Add 135  $\mu\text{L}$  of flashPAGE Filter Binding Additive to the Lower Running Buffer (which contains the small nucleic acids after flashPAGE fractionation) and mix by pipetting up and down several times.

## 4. Add 1.1 mL of 100% ethanol and mix well.

Add 1.1 mL of 100% ethanol to the mixture from the previous step and mix by pipetting up and down several times.

## 5. Draw the mixture through the prewet Filter Cartridge.

The total volume of the nucleic acid mixture at this point will be almost 1.5 mL. Draw the mixture through a prewet Filter Cartridge (from step [1](#)) either by 3 successive centrifugations or using a vacuum manifold:

### Centrifuge users:

- a. Pipet  $\sim$ 500  $\mu\text{L}$  of the mixture from step [4](#) onto a prewet Filter Cartridge/Tube and centrifuge at  $\sim$ 10,000 X g to pass the mixture through the filter ( $\sim$ 15 sec to 1 min).
- b. Discard the flow-through and repeat two more times to pass the entire sample through the filter.

**Vacuum manifold users:**

- a. Load the vacuum manifold with a 3 mL syringe barrel and a prewet Filter Cartridge (from step [1](#)), and apply the vacuum.
- b. Pipet the mixture from step [4](#) onto the Filter Cartridge. The vacuum will draw it through the filter.

**6. Wash with 2 x 500  $\mu$ L flashPAGE Wash Solution**

Make sure that the ethanol has been added to the flashPAGE Wash Solution Concentrate before using it.

- a. Add 500  $\mu$ L flashPAGE Wash Solution to the Filter Cartridge and draw it through the filter as in the previous step. Discard wash solution.
- b. Repeat with a second 500  $\mu$ L of flashPAGE Wash Solution.
- c. After discarding the flashPAGE Wash Solution, centrifuge the Filter Cartridge/Tube for 1 min at  $\sim 10,000 \times g$  to remove the last traces of liquid from the filter.

**7. Elute small nucleic acids by applying 2 x 15  $\mu$ L hot Nuclease-free Water and incubating for 5–10 min at 60–70°C**

The minimum elution volume is 2 x 15  $\mu$ L, but more eluent can be used if desired.

- a. Place the Filter Cartridge into an Elution Tube.
- b. Apply 15  $\mu$ L of 95°C Nuclease-free Water to the center of the filter in the Filter Cartridge. Close the cap of the tube and incubate at 60–70°C for 5–10 min.
- c. Recover eluted nucleic acids by centrifuging at 10,000  $\times g$  for 1 min at room temp.
- d. Repeat with a second 15  $\mu$ L aliquot of 95°C Nuclease-free Water. Collect the eluate into the same tube.

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**F. Quality Control****Functional testing**

The flashPAGE Reaction Clean-Up Kit is functionally tested for recovery of 20 nt and 30 nt radiolabeled RNA species spiked into a total RNA sample.

## **Nuclease testing**

Relevant kit components are tested in the following nuclease assays:

### **RNase activity**

Meets or exceeds specification when a sample is incubated with labeled RNA and analyzed by PAGE.

### **Nonspecific endonuclease activity**

Meets or exceeds specification when a sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

### **Exonuclease activity**

Meets or exceeds specification when a sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

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## **G. Safety Information**

### **Chemical safety guidelines**

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety goggles, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

## About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

## Obtaining the MSDS

To obtain Material Safety Data Sheets (MSDSs) for any chemical product supplied by Applied Biosystems or Ambion:

- At [www.appliedbiosystems.com](http://www.appliedbiosystems.com), select **Support**, then **MSDS**. Search by chemical name, product name, product part number, or MSDS part number. Right-click to print or download the MSDS of interest.
- At [www.ambion.com](http://www.ambion.com), go to the web catalog page for the product of interest. Click **MSDS**, then right-click to print or download.
- E-mail ([MSDS\\_Inquiry\\_CCRM@appliedbiosystems.com](mailto:MSDS_Inquiry_CCRM@appliedbiosystems.com)) or telephone (650-554-2756; USA) your request, specifying the catalog or part number(s) and the name of the product(s). We will e-mail the associated MSDSs unless you request fax or postal delivery. Requests for postal delivery require 1–2 weeks for processing.

For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.



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