

Time Course for Fractionation of Nucleic Acids with the flashPAGE™ Fractionator



version 0503

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With some experimentation, the flashPAGE Fractionator can be used to isolate any size class of single-stranded nucleic acids in the 15-100 nt range. By collecting the Lower Running Buffer and replacing it with fresh running buffer, a single flashPAGE gel can fractionate a complex sample mixture into multiple fractions based on their size.

Maintaining consistent fractionation between runs

Observe the following recommendations to maintain as much consistency as possible between runs:

- Use the same electrophoresis power supply; slight variations between power supplies can alter relative migration rates.
- Use the same voltage setting.
- Use the same sample load volume.
- Load the same mass amount of nucleic acid.
- Samples should always have the same salt concentration.
- Always remove excess gel storage buffer from the flashPAGE Pre-Cast Gel cartridges before loading into the flashPAGE Fractionator. With such small volumes of Upper and Lower Running Buffers, any addition of the gel storage buffer will affect sample migration rate.

The flashPAGE Gel loading Buffer A40 migrates with 40 nt nucleic acids. To identify an approximate time for elution of nucleic acid size fractions other than 15-40 nt, we suggest the following preliminary calibration experiment. Prepare a sample that is easily obtained that will closely mimic your experimental sample. We highly recommend using radiolabeled Decade Markers from Ambion (Cat #7776) as a tracer in a mock sample mix for this purpose. By collecting the Lower Running Buffer and replacing it several times at set timepoints, you can determine at what time point differently sized nucleic acids will exit the flashPAGE gel.

Time Course

1. Follow the flashPAGE instruction card for fractionator Set-Up and Sample Preparation and Loading.
2. Start a timer when beginning electrophoresis.
3. Collect Lower Running Buffer fractions over a time course.
 - Use the migration of the dye in the flashPAGE Gel Loading Buffer A40, which migrates with 40 nt nucleic acids, as a guide for determining when to start collecting Lower Running Buffer. If the desired size fraction is larger than 40 nt, begin collecting Lower Running Buffer when the blue dye begins to exit the gel.
 - Stop electrophoresis by opening the lid of the flashPAGE Fractionator. Remember to stop the timer each time you stop the electrophoresis.
 - Use a pipette to transfer the Lower Running Buffer out of the lower buffer chamber into a clean 1.5 ml microtube.
 - Store the fractions on ice (storage at elevated temperatures can cause degradation of the sample).
4. Replace the Lower Running Buffer and continue the time course at regular intervals (e.g., 1-3 min), collecting the Lower Running Buffer at each time point.
5. Desalt, concentrate, and analyze equivalent amounts of the collected fractions by PAGE (see Figure 1 below). Run an equivalent amount of unfractionated sample on the gel for comparison. Samples can be desalted and concentrated by overnight sodium acetate/ethanol precipitation (see www.ambion.com/prod.flashPAGE for a protocol), or by using the flashPAGE Reaction Clean-Up Kit (Cat #12200).
6. Evaluate the size of nucleic acids collected at each time point. By using identical conditions for fractionation, nucleic acids size fractions will exit the flashPAGE gel at the same timepoint during each run.

Figure 1. Time Course to Fractionate Decade Markers on a flashPAGE Pre-Cast

