Lonza

FlashGel™ System

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FlashGel™ System
Important Safety Information
Safety symbols

The following symbols alert the user to important operational, maintenance, and/or warranty requirements, or possible hazards exposure.

Symbol indicates a general caution or warning that the user may be aware of. Including but not limited to – potential chemical or hazardous light exposure.

Symbol indicates a warning of potential exposure to hazardous voltage where contact may result in death or serious injury.

General Product Warning Statement:

CAUTION: Hazardous Voltage
Contact may cause death or serious injury

Caution should be exercised in the operation of this system as it can develop sufficient voltage and current to produce a lethal shock. To avoid any risk of injury, the system should only be operated by properly trained personnel and always in accordance with the instructions provided.

Prior to turning on the DC power source, ensure that the black lead is connected to the negative terminal and the red lead is connected to the positive terminal. Do not touch the FlashGel™ Dock or Cassette while the high voltage supply is turned on. Do not flood wells, add samples, or extract bands while the high voltage leads are connected to the power supply.

Failure to adhere to these instructions could result in personal and /or laboratory hazards, as well as invalidate any warranty. Always turn off the DC power source prior to removing cassettes from the dock. For maximum safety, always operate this system in an isolated, low traffic area not accessible to unauthorized personnel. Never operate damaged or broken equipment.

Precautions

The FlashGel™ Dock utilizes Dark Reader® Transilluminator technology (Clare Chemical Research, Inc.) to view fragments. It is safe to view cassettes on the lighted dock without UV light protection. Turn on the light only after the cassette is in place. Do not stare directly into the light.

> ⚠ **CAUTION**: Use the FlashGel™ Mask to block light from the second tier of wells when using double-tier or FlashGel™Recovery Cassettes.

Wear gloves, lab coat, and safety glasses when handling FlashGel™ Cassettes. The gel and buffer in FlashGel™ Cassettes contain a proprietary nucleic acid gel stain that is a potential mutagen. Follow state

> ⚠ **CAUTION:** To avoid accidental exposure to high voltage, do not reverse electrodes in order to run samples backwards.

and local guidelines for handling and disposal of these materials.

Operating conditions for the FlashGel™ Dock

Maximum Limits

Electrophoresis unit: 0 - 300 VDC high voltage DC input
15 watts power
50 mA current
Dock light: 18 VDC low voltage DC input

Environmental Conditions

Operating Conditions:

- Temperature: 15°C-35°C
- Humidity: 15%-85% relative humidity, non-condensing
- For indoor use only
- Altitude up to 2000m

Storage and Shipping Conditions (FlashGel™ Dock)

- Temperature: 2°C-60°C
- Humidity: 15%-85% relative humidity, non-condensing

Cleaning, Maintenance and Disposal

Cleaning procedure

CAUTION: To avoid accidental exposure to high voltage, do not clean dock while connected to the high voltage power supply.

Clean the FlashGel™ Dock with a cloth moistened with water or mild detergent. Do not immerse!

Maintenance

Visually inspect the dock prior to use for signs of wear, cracks or damage. Do not use if damage is found.

There are no user serviceable parts contained in the FlashGel™ Dock.

Disposal

The stain in the FlashGel™ Cassettes is a potential mutagen. Follow state and local guidelines for disposal of these materials.

Specifications

Separation Range:

1.2% DNA Cassettes:	50 bp – 4 kb (up to 10 kb with longer run times)
2.2% DNA Cassettes:	10 bp – 1 kb
1.2% RNA Cassettes:	0.5 kb – 9 kb
1.2% Recovery Cassettes:	50 bp – 4 kb
2.2% Recovery Cassettes:	10 bp – 1 kb

Cassette Storage:

DNA Cassettes:	18°C-26°C for 5 months from date of manufacture
RNA Cassettes:	Please inquire
Recovery Cassettes:	18°C-26°C for 5 months from date of manufacture

Well Volume:

12 + 1 wells:	Do not exceed 5 µl sample/well
16 + 1 wells:	Do not exceed 5 µl sample/well
8 + 1 wells:	Do not exceed 12 µl sample/well

Gel Size:

70 mm (L) x 84 mm (W) x 2 mm (H)

Cassette Size:
115 mm (L) x 107 mm (W) x 17 mm (H)

Dock Size:
134 mm (L) x 120 mm (W) x 54 mm (H)

Cassette Contents:
Agarose gel, stain and buffer

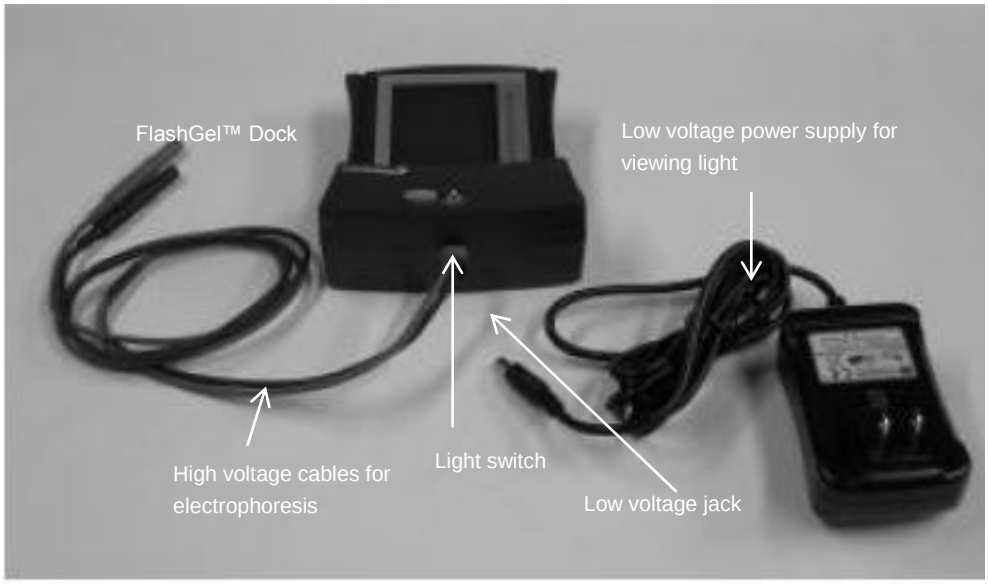
Equipment Ratings

Electrophoresis input (high voltage DC):
Voltage: 0 - 300 VDC
Power: 15 W
Current: 50 mA

Dock light input (low voltage DC):
Voltage: 18 VDC
Current: 1.11 A

Dock light transformer input (line voltage AC):
Voltage: 100-240 VAC, 50-60 Hz
Current: 1.0 A

Electrical Connections:
High voltage (electrophoresis): shielded, retractable banana plugs
Low voltage (light): 2.1 x 5.5 x 14 mm jack



FlashGel™ System Quick Start Guide

Important Points

- Do not exceed 5 μl sample volume per lane for 12 + 1 well and 16 + 1 well cassettes; or 12 μl sample volume per lane for 8 + 1 well cassettes.

- Optimal sample concentrations are approximately 1/5 the typical per band concentration of an ethidium bromide gel.

- For best results flood sample wells with water prior to loading, and use FlashGel™ Loading Dye and FlashGel™ Markers, and FlashGel™ Recovery Buffer.

- Use the FlashGel™ Mask when running double-tier cassettes.

- Use the FlashGel™ Visualization Glasses when recovering samples.

Instructions

1. Refer to Table 1 (pp 8-9) for recommended sample preparation and run conditions.

2. Remove white well seals from cassette. Do not remove the clear side vent seals.

3. Flood sample wells with distilled or deionized water. Tilt cassette to move excess fluid to the edge and blot off with a lint free wipe. Do not blot wells directly.

4. Insert cassette into dock. Insert FlashGel™ Mask under the central tier of sample wells if using double-tier or recovery cassettes.

5. Load samples. Samples to be recovered should be loaded in upper tier sample wells.

6. Plug in high voltage cables, turn on power supply and set to recommended voltage.

7. Plug in low voltage power supply and turn on light.

8. If using standard DNA and RNA cassettes run for recommended time or until separation of desired fragments is complete. If using recovery cassettes run and observe migration of sample; just prior to desired sample reaching recovery wells (2nd-tier) stop the run and disconnect the high voltage cables (complete steps 9 – 13).

9. Blot excess buffer from the recovery well(s) and add 20 μl of FlashGel™ Recovery Buffer.

10. Remove FlashGel™ Mask, reconnect voltage cables, and restart power. Use FlashGel™ Visualization Glasses to observe band migration.

11. When the band of interest has migrated to the center of the recovery well, turn off power supply and disconnect voltage cables. Use a pipette to carefully remove the recovery buffer (containing the DNA) from the recovery well.

12. If necessary, the process (addition of recovery buffer, electrophoresis, and recovery) may be repeated to increase recovery of higher DNA loads.

13. Photograph using The FlashGel™ Camera, or other standard camera and transilluminator.

Table 1. Recommended Sample Preparation and Run Conditions

	DNA Cassettes	RNA Cassettes	Recovery Cassettes
Separation range	1.2%: 50 bp – 10 kb 2.2%: 10 bp – 1 kb Separation of fragments > 4 kb will be improved by running longer at lower voltage	1.2%: 0.5 kb – 9 kb	1.2%: 50 bp – 10 kb 2.2%: 10 bp – 1 kb Separation of fragments > 4 kb will be improved by running longer at lower voltage
Sample preparation	For best results, dilute DNA samples in 1X FlashGel™ Loading Dye	Denatured RNA samples: Prepare samples in 50% formaldehyde sample buffer and RNase-free water; denature for 5 minutes at 65°C. Native RNA samples: Use FlashGel™ Loading Dye	For best results, dilute DNA samples in 1X FlashGel™ Loading Dye
Sample concentration and detection limits	Optimal DNA load levels are 5-20 ng/band in a 5 µl load; for best results, do not exceed 20 ng/band	Optimal RNA load levels will vary depending upon RNA sample; for best results, do not exceed 200 ng/band in a 5 µl load	Optimal DNA load levels are 50-500 ng/band in load volumes up to 12 µl
Voltage & run time	Single-tier: 275 V for 2-7 minutes Double-tier: 275 V for 2-5 minutes	Single-tier: 225 V for 4-8 minutes Double-tier: 225 V for 3-5 minutes	275 V for time needed to electrophorese bands to recovery wells – varies by fragment size from 3+ minutes Maximum run time 12-14 minutes
Recovered concentration and volume	N/A	N/A	Sample recoveries are typically 80-90%, depending upon fragment Recovery volumes are typically 15-50 µl

	DNA Cassettes	RNA Cassettes	Recovery Cassettes
Recommended Markers	1.2% cassettes: FlashGel™ DNA Marker 100 bp–4 kb 2.2% cassettes: FlashGel™ DNA Marker 50 bp – 1.5 kb Double-tier cassettes: FlashGel™ DNA Marker 100 bp – 3 kb	FlashGel™ RNA Marker 0.5 kb–9 kb	FlashGel™ DNA Marker 100 bp–3 kb FlashGel™ QuantLadder 100 bp-3 kb 2.2% cassettes: FlashGel™ DNA Marker 50 bp – 1.5 kb

FlashGel™ System for DNA Instructions

Introduction

The FlashGel™ System is recommended for fast separation and analysis of DNA.

FlashGel™ DNA Cassettes:

- Sizing and/or quantitation of PCR or restriction fragments 10 bp – 10 kb
- Confirmation of PCR amplification

Separation of DNA fragments can be monitored in real time at the lab bench without the use of UV illumination.

Fragments separated with the FlashGel™ System may be photographed with The FlashGel™ Camera, or other standard documentation systems.

FlashGel™ DNA Cassettes are not recommended for recovery. Use FlashGel™ Recovery Cassettes (page 25) for DNA to be recovered.

Important Information

Run conditions and resolution

The FlashGel™ System is designed for fast, high voltage separation of fragments 10 bp to 10 kb. Cassettes may be run at lower voltages for longer times to improve separation of fragments > 4 kb (page 18). Monitor the run and optimize conditions. Fragments will run faster on a warm dock. Reduce run time, or lower voltage as necessary. Refer to Table 1 (pp 8-9) for recommended run conditions.

Visualization of bands on the FlashGel™ Dock

DNA and marker bands will be visible on the lighted dock under typical lab lighting. The degree of visibility may vary based upon the overall light intensity in the lab. Bands are best viewed looking straight down at the dock in a space that minimizes light intensity and reflection.

Alternatively, band separation may be visualized on a computer screen with the aid of the FlashGel™ Camera.

To ensure adequate visibility of a marker for monitoring the run on the dock, use FlashGel™ DNA Markers or FlashGel™ QuantLadders. Refer to

Table 2 (pp 14) and Figure 2 (page 16) for details on DNA concentrations visible under different conditions.

DNA bands are visible on the dock throughout the run.

Loading dye and markers

The FlashGel™ System is compatible with standard gel loading dyes and markers. Bromophenol blue dye does not migrate in FlashGel™ Cassettes; however samples containing bromophenol blue may be used, as sample migration is not affected. Use the FlashGel™ Loading Dye and FlashGel™ Markers or FlashGel™ QuantLadder for best results.

Preparing the gel for running

For best results, use a transfer pipette or squirt bottle to flood sample wells with distilled or deionized water before loading samples or markers. This will ensure adequate moisture in the wells. Flood the wells, then tilt the cassette and use a lint free wipe or small piece of blotting paper to remove excess liquid from the cassette. Do not blot the wells directly.

DNA sensitivity levels

The FlashGel™ System uses a proprietary stain that is 5–20 times more sensitive than ethidium bromide stain. In general, use per band DNA concentrations 1/5 of those normally used for ethidium bromide detection.

Optimal DNA load levels on FlashGel™ Cassettes are 5-20 ng per band. DNA levels below 5 ng per band may not be visible on the dock, but levels as low as 0.10 ng per band can be detected on gel images/photos. DNA levels up to 80 ng per band can be used; however, levels exceeding 20 ng per band may result in band distortion. DNA levels may be adjusted to provide best performance for the image analysis system used.

Because of the sensitivity of the FlashGel™ System, most DNA samples should be diluted, and load volume per well must not exceed 5 μl.

For best results, dilute DNA samples into 1X FlashGel™ Loading Dye such that a 5 μl sample contains 5-10 ng DNA per band.

Gel Running Instructions

> ⚠**CAUTION:** Wear gloves, lab coat and safety glasses when handling FlashGel™ Cassettes.

1. Prepare samples. Refer to Table 1 (pp 8-9) for recommended sample preparation.

2. Tear open the pouch and remove the cassette.

   NOTE: If cassette is wet, dry with a clean wipe.

   NOTE: Air pockets may be present between the gel and cassette. These will not affect band migration.

3. Place the cassette on a flat surface and pull the tab to remove the white well seal. Do not remove the clear seals covering the side vent holes.

4. Flood all sample wells with distilled or deionized water. Tilt the cassette and use a lint free wipe or small piece of blotting paper to remove excess fluid. Do not blot the wells directly.

> ⚠**CAUTION:** To avoid accidental exposure to high voltage, do not flood wells while cassette is connected to the high voltage power supply.

   NOTE: For best results, flood wells with AccuGENE™ Molecular Biology Water (Cat. #51200).

5. Place the cassette on the dock and slide it into place; the cassette should snap securely into the dock.

6. Load samples and markers. Refer to Table 1 (pp 8-9) for sample preparation and recommended markers.

> **CAUTION:** To avoid accidental exposure to high voltage, do not load samples while cassette is connected to the high voltage power supply.

7. Connect the low voltage power supply to the dock by inserting the lead into the receptacle at the back of the dock and plugging in the power supply. This is the power supply to the light source.

8. Turn on the dock light by pressing the orange button on the top of the unit.

   NOTE: The light will automatically shut off after 10 minutes. Re-start by pressing the orange button.

9. Connect the high voltage leads to power supply and set power at recommended voltage (Table 1, pp 8-9).

   NOTE: Typical starting currents should range from 20-25 mA for DNA gels. Run until desired separation is obtained for the fragments of interest. Under the conditions outlined, DNA fragments of 50 bp-100 bp will migrate out of the gel in 7-8 minutes. Refer to Figure 1 (page 15) for separation results at various run times.

   NOTE: Running multiple cassettes in quick succession may require slight adjustment of running conditions as bands will run faster as the dock gets warmer. Monitor separation and reduce voltage and run time if necessary.

   NOTE: Some expression of buffer from the wells is normal.

> **CAUTION:** Handle cassettes only after voltage is turned off and leads are disconnected.

10. Run DNA cassettes until desired separation is reached for the fragment of interest.

11. Turn off voltage and disconnect leads from the power supply prior to removing the cassette.

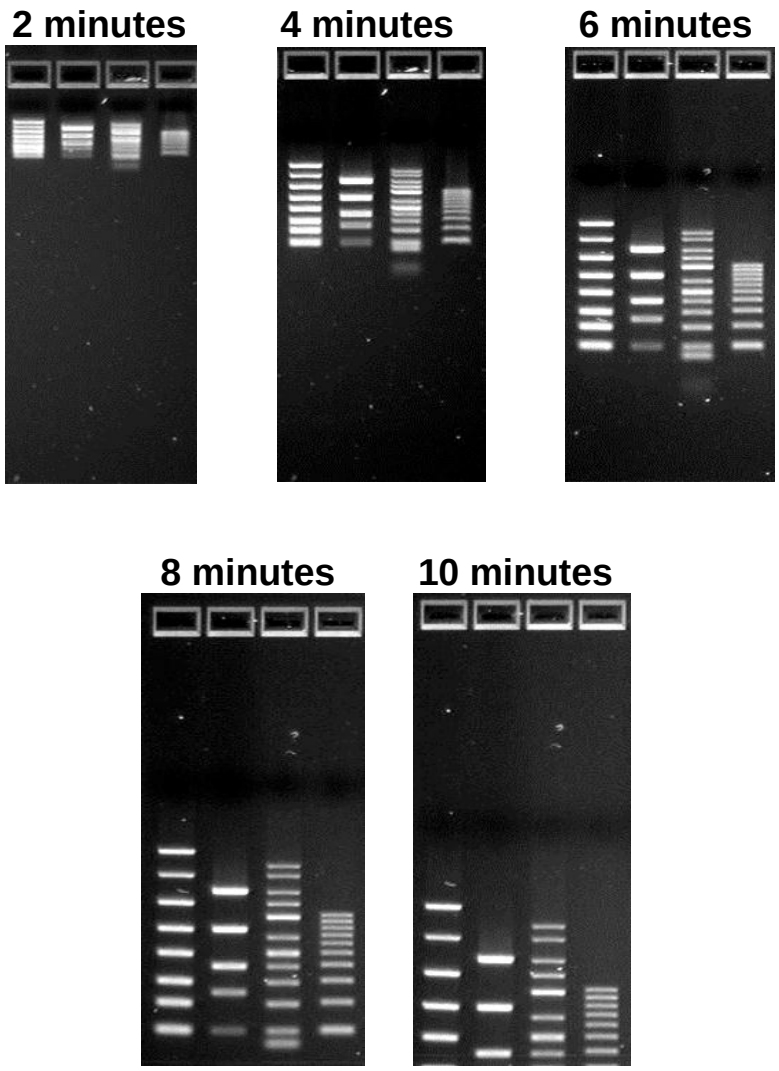
12. Record the gel data using The FlashGel™ Camera, by Polaroid® photography, or by other image capture systems. See Figure 2 (page 16) for details on typical cameras.

Reference Information

Table 2. DNA Amount Visible on FlashGel™ DNA Cassettes Under Various Conditions

	1500 bp Fragment	400 bp Fragment
Viewed in ambient light	1.6 – 3.2 ng	1.6 – 3.2 ng
Viewed in Dark Room	0.39 – 0.78 ng	0.39 – 0.78 ng
Viewed on FlashGel™ Camera	0.10 – 0.20 ng	0.10 – 0.20 ng
FlashGel™ Camera Photo	0.10 – 0.20 ng	0.10 – 0.20 ng
Viewed on Dark Reader® Transilluminator	0.05 – 0.01 ng	0.10 – 0.20 ng
Dark Reader® Photo	0.10 – 0.20 ng	0.10 – 0.20 ng
Viewed on UV	0.39 – 0.78 ng	0.39 – 0.78 ng
UV Photo	0.39 – 0.78 ng	0.39 – 0.78 ng

Fig 1. Examples of Separation at Various Run Times at 275 V on a FlashGel™ DNA Cassette (1.2%, 12+1 well single-tier)



Sample lanes left to right:

Lane 1. **FlashGel™ DNA Marker 100/200/300/500/800/1250/2000/4000 bp** (5 μl load 1:5 dilution)

Lane 2. FlashGel™ QuantLadder 100/250/400/800/1500 bp (5 μl load 1:5 dilution)

Lane 3. Lonza Marker 50 bp – 2500 bp (3 μl load 1:5 dilution)

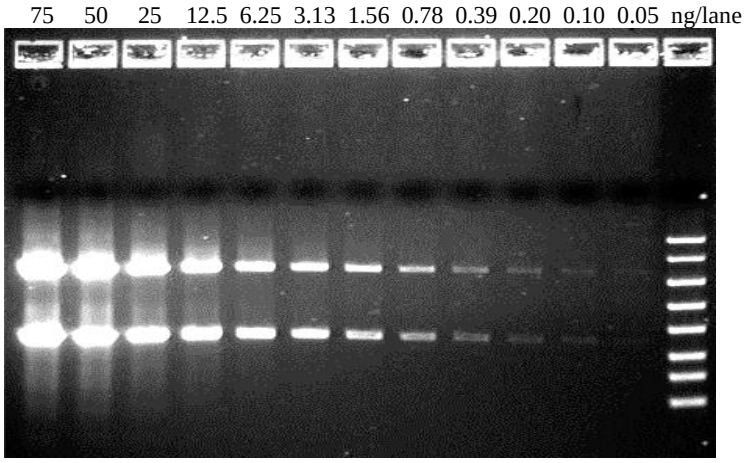
Lane 4. Lonza Ladder 100 bp (3 μl load 1:15 dilution)

Fig 2. Detection of DNA Fragments on FlashGel™ DNA Cassettes

- Illuminate cassettes using UV or blue-light transilluminators, such as the Dark Reader® Transilluminator.
- Photograph using FlashGel™ Camera, or any system used for standard ethidium bromide stained gels.
- For Polaroid® Type 57 film with a UV transilluminator, start with 1-2 second exposures.
- For CCD systems, use the system's existing ethidium bromide filter.
- FlashGel™ DNA Marker (100 bp - 4 kb) is loaded in far right lane.

Images below show a dilution series of 400 bp and 1500 bp fragments

Dark Reader® Transilluminator with orange cover, CCD Camera with EtBr Filter, 3 second exposure



UV transilluminator, Polaroid® Camera with EtBr Filter, 3 second exposure

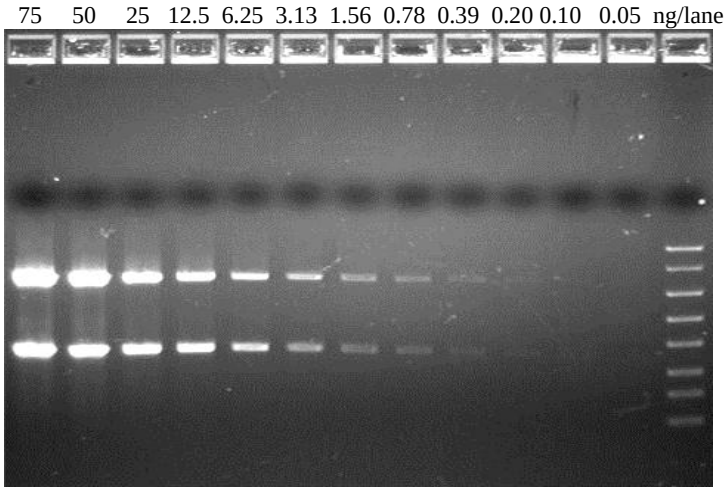
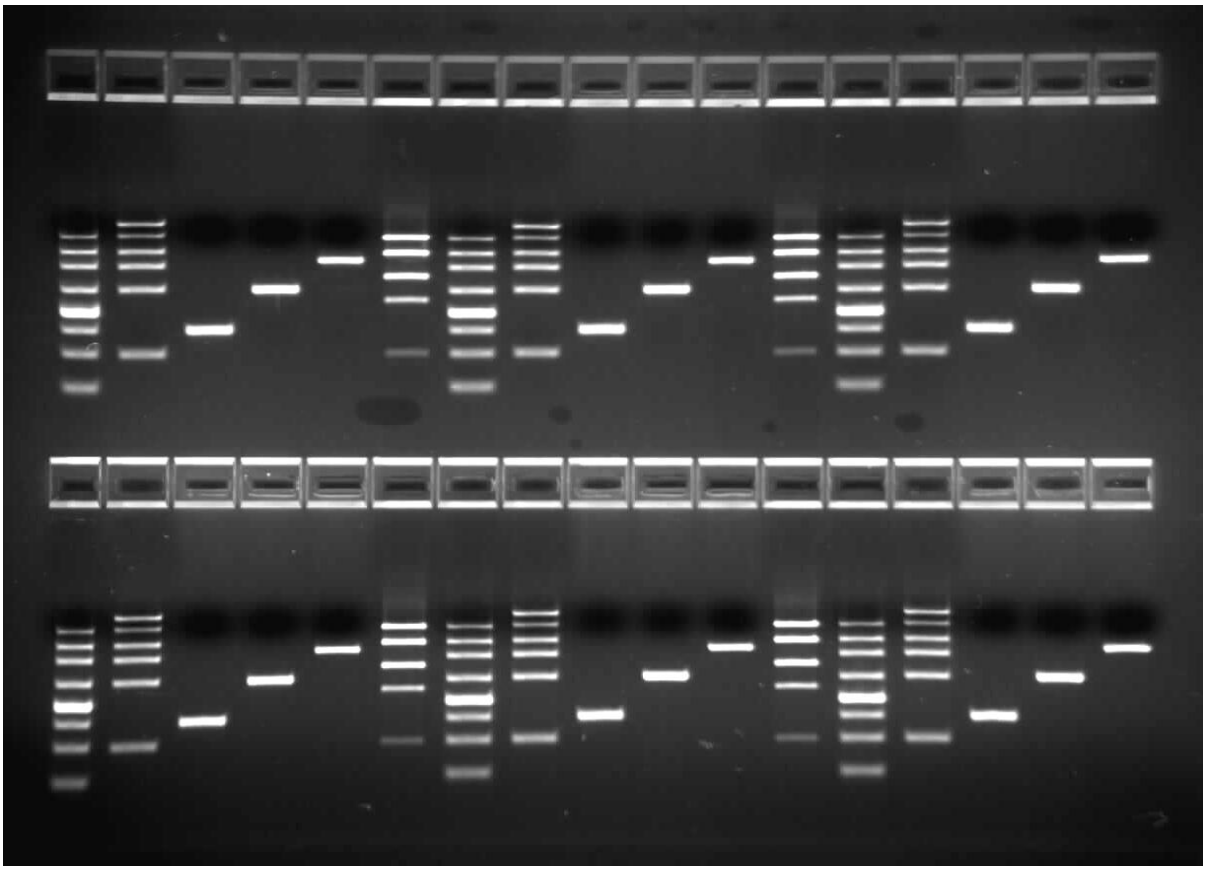


Fig. 3. Separation of Markers and DNA Fragments on a FlashGel™ DNA Cassette (2.2%, 16 + 1 well double-tier)



Sample lanes:

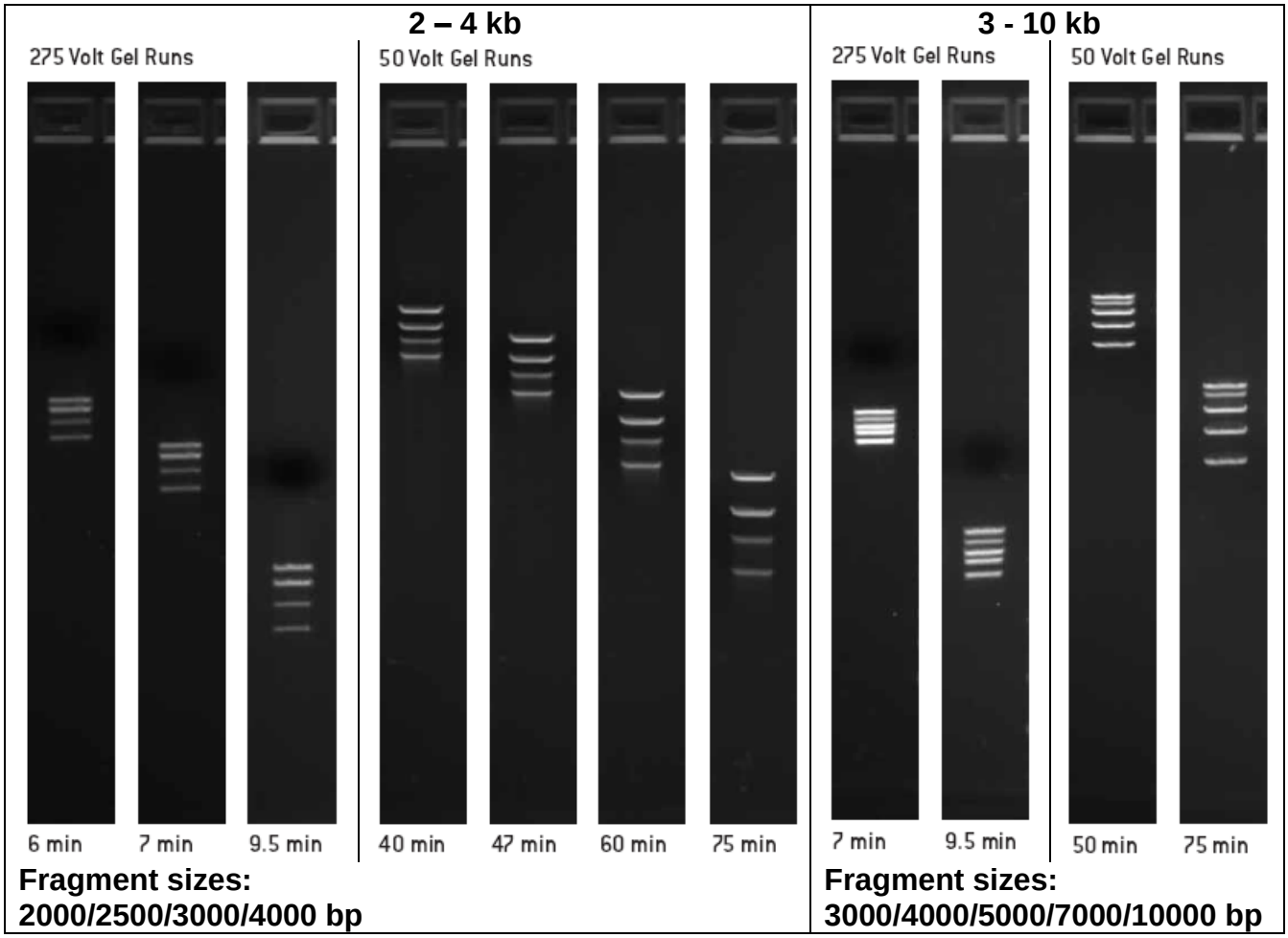
Lanes 1, 7, 13: FlashGel™ 50 bp – 1.5 kb Marker
(recommended for 2.2% cassettes)

Lanes 2, 8, 14: FlashGel™ 100 bp – 3 kb Marker
(recommended for double-tier cassettes)

Lanes 6 and 12: FlashGel™ QuantLadder

Lanes 3, 4, 5/9, 10, 11/15, 16, 17: 8 ng loads of 150 bp, 300 bp and 800 bp DNA fragments

Fig. 4. Improving Large DNA Fragment Separation on the FlashGel™ System



FlashGel™ System for RNA Instructions

Introduction

The FlashGel™ System is recommended for fast separation and analysis of RNA, including:

- Verification and analysis of total RNA 0.5 kb – 9 kb
- Checking for RNA degradation
- Quick checks of native RNA

Fragments separated with the FlashGel™ System may be photographed using the FlashGel™ Camera or other standard documentation systems.

FlashGel™ RNA Cassettes are not recommended for recovery.

Important Information

Run conditions and resolution

The FlashGel™ System is designed for fast, high voltage separations. Cassettes may be run at lower voltages for longer times to improve separation of higher molecular weight fragments. Monitor the run and optimize conditions. Fragments will run faster on a warm dock. Reduce run time, or lower voltage as necessary. Refer to Table 1 (pp 8-9) for recommended run conditions.

Visualization of bands on the FlashGel™ Dock

RNA and marker bands will be visible on the lighted dock under typical lab lighting. The degree of visibility may vary based upon the overall light intensity in the lab. Bands are best viewed looking straight down at the dock in a space that minimizes light intensity and reflection.

RNA bands will be visible on the dock for the first 3-4 minutes of the run, after which they fade and then reappear after a $\geq$ 10 minute post-run hold period. The FlashGel™ DNA Marker may be used to monitor the RNA run.

Loading dye and markers

The FlashGel™ System for RNA is compatible with standard formaldehyde loading dye and RNA markers. Bromophenol blue dye does not migrate in FlashGel™ Cassettes; however samples containing bromophenol blue may

be used, as sample migration is not affected. Use the Lonza Formaldehyde Sample Buffer or FlashGel™ Loading Dye (for native RNA), and FlashGel™ RNA Marker for best results.

Preparing the gel for running

For best results, use a transfer pipette or squirt bottle to flood sample wells with RNase-free water before loading samples or markers. This will ensure adequate moisture in the wells. Flood the wells, then tilt the cassette and use a lint free wipe or small piece of blotting paper to remove excess liquid from the cassette. Do not blot the wells directly.

RNA sensitivity levels

The FlashGel™ System uses a proprietary stain that is 5–20 times more sensitive then ethidium bromide stain and will detect RNA quantities < 10 ng per band.

For denatured RNA, dilute samples with formaldehyde loading buffer such that a 5 µl load contains ≤ 200 ng RNA per band.

For native RNA, dilute samples into FlashGel™ Loading Dye such that a 5 µl load contains ≤ 200 ng RNA per band.

Gel Running Instructions

> ⚠**CAUTION:** Wear gloves, lab coat and safety glasses when handling FlashGel™ Cassettes.

1. Prepare samples. Refer to Table 1 (pp 8-9) for recommended sample preparation.

2. Tear open the pouch and remove the cassette.

   NOTE: If cassette is wet, dry with a clean wipe.

   NOTE: Air pockets may be present between the gel and cassette. These will not affect band migration.

3. Place the cassette on a flat surface and pull the tab to remove the white well seal. Do not remove the clear seals covering the side vent holes.

4. Flood all sample wells with RNase-free water. Tilt the cassette and use a lint free wipe or small piece of blotting paper to remove excess fluid. Do not blot the wells directly.

   NOTE: For best results, flood wells with AccuGENE™ Molecular Biology Water (Cat. #51200).

> ⚠ **CAUTION:** To avoid accidental exposure to high voltage, do not flood wells while cassette is connected to the high voltage power supply.

5. Place the cassette on the dock and slide it into place; the cassette should snap securely into the dock.

> ⚠ **CAUTION:** To avoid accidental exposure to high voltage, do not load samples while cassette is connected to the high voltage power supply.

6. Load samples and markers. Refer to Table 1 (pp 8-9) for sample preparation and recommended markers.

> ⚠ **CAUTION**: Use the FlashGel™ Mask to block light from the second tier of wells when using double-tier cassettes.

7. Connect the low voltage power supply to the dock by inserting the lead into the receptacle at the back of the dock and plugging in the power supply. This is the power supply to the light source.

8. Turn on the dock light by pressing the orange button on the top of the unit.

   NOTE: The light will automatically shut off after 10 minutes. Re-start by pressing the orange button.

9. Connect the high voltage leads to power supply and set power at recommended voltage (Table 1, pp 8-9).

NOTE: Typical starting currents should be 25 - 30 mA. Run until desired separation is obtained for the fragments of interest.

NOTE: Running multiple cassettes in quick succession may require slight adjustment of running conditions as bands will run faster as the dock gets warmer. Monitor separation and reduce voltage and run time if necessary.

NOTE: Some expression of buffer from the wells during run is normal.

10. Run RNA cassettes for 8 minutes, then turn off voltage and disconnect leads from the power supply prior to removing the cassette.

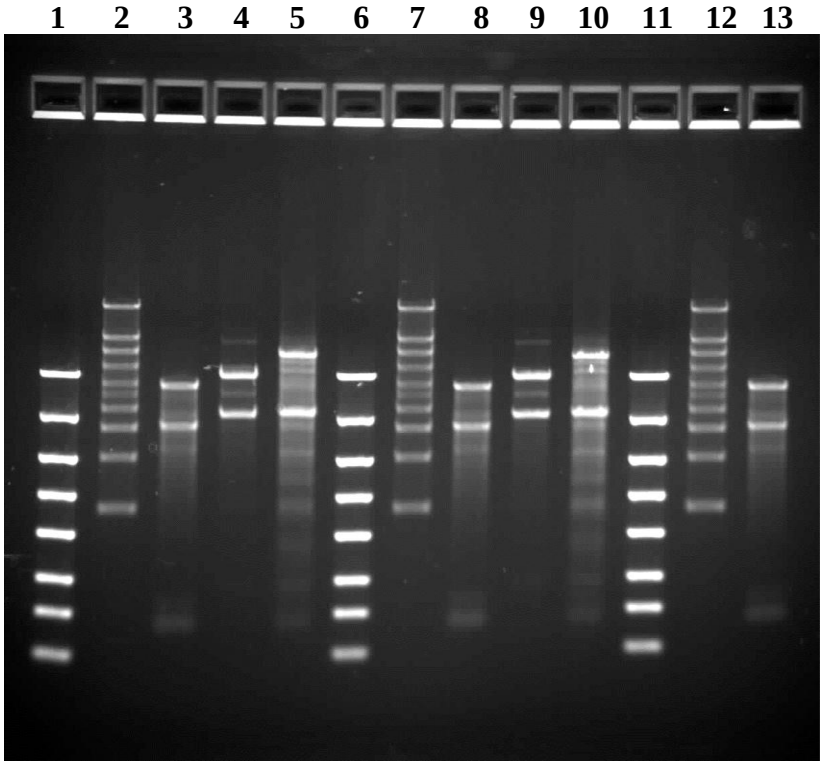
> ⚠ **CAUTION:** Handle cassettes only after voltage is turned off and leads are disconnected.

11. Remove the cassette and let it stand at room temperature for ≥10 minutes, or until the fragments are visible at the desired intensity. Maximum intensity is reached in approximately 45 minutes.

Record the gel data using The FlashGel™ Camera, or by Polaroid® photography or other image capture systems. See Figures 5 and 6 (pp 23-24) for RNA images.

Reference Information

Fig. 5. Detection of RNA Fragments on a FlashGel™ RNA Cassette



Cassette run for 8 minutes at 225 V; photographed 20 minutes post-run.

Sample Lanes:

Lanes 1, 6, 11: DNA Marker (for visualization during run)

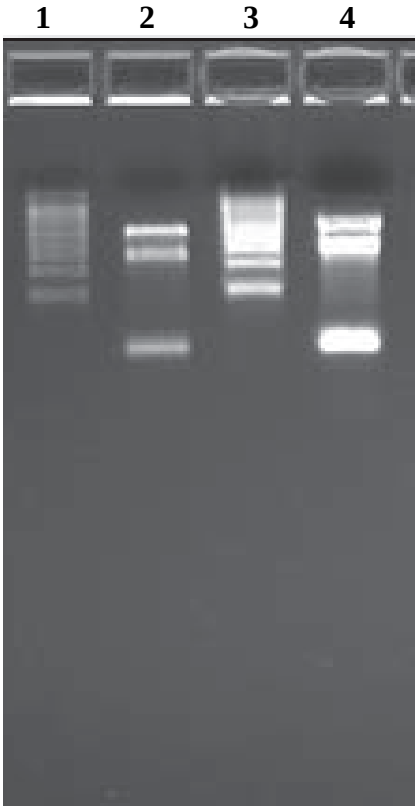
Lanes 2, 7, 12: 100 ng RNA Ladder

Lanes 3, 8, 13: 100 ng *E. coli* Total RNA (Ambion)

Lanes 4, 9: ~100 ng *S. cervevisiae* Total RNA purified using RiboPure™ Yeast Kit (Ambion)

Lanes 5, 10: 100 ng Mouse Thymus Total RNA (Ambion)

Fig. 6. Quick Check of Native RNA Fragments on a FlashGel™ RNA Cassette (1.2%, 12+1 single-tier)



Cassette run for 4 minutes at 225 V; followed by immediate imaging.

Sample lanes:

Lane 1: Lonza Marker, 50 ng

Lane 2: *E. Coli* Total RNA, 50 ng

Lane 3: Lonza Marker, 250 ng

Lane 4: *E. Coli* Total RNA, 250 ng

FlashGel™ System for Recovery Instructions

Introduction

The FlashGel™ System for Recovery is recommended for fast separation and recovery of sheared DNA and fragments from 10 bp – 4 kb.

Separation of DNA fragments can be monitored in real time and fragments can be recovered at the lab bench without the use of UV illumination, or the need for band excision.

Fragments separated with the FlashGel™ Recovery System are compatible with standard molecular biology applications (PCR amplification, ligation and cloning, etc.)

Important Information

Run conditions and resolution

The FlashGel™ System for Recovery is designed for fast, high voltage separations and recovery. Cassettes may be run at lower voltages for longer times to improve separation of larger fragments. Monitor the run and optimize conditions for the fragment of interest. Fragments will run faster on a warm dock. Reduce run time, or lower voltage as necessary. Do not exceed 14 minutes total run time. Refer to Table 1 (pp 8-9) for recommended run conditions.

Visualization of bands on the FlashGel™ Dock

DNA and marker bands will be visible on the lighted dock under typical lab lighting. The degree of visibility may vary based upon the overall light intensity in the lab. Bands are best viewed looking straight down at the dock in a space that minimizes light intensity and reflection.

To ensure adequate visibility of a marker for monitoring the run, use FlashGel™ DNA Markers or FlashGel™ QuantLadders. Refer to Table 2 (page 14) for details on DNA concentrations visible under different conditions.

Loading dye and markers

The FlashGel™ System is compatible with standard gel loading dyes and markers. Bromophenol blue dye does not migrate in FlashGel™ Cassettes; however samples containing bromophenol blue may be used,

as sample migration is not affected. Use the FlashGel™ Loading Dye and FlashGel™ Markers or FlashGel™ QuantLadder for best results.

Preparing the gel for running

For best results, use a transfer pipette or squirt bottle to flood sample wells with distilled or deionized water before loading samples or markers. This will ensure adequate moisture in the wells. Flood the wells, then tilt the cassette and use a lint free wipe or small piece of blotting paper to remove excess liquid from the cassette. Do not blot the wells directly.

For best results, dilute DNA sample into 1X FlashGel™ Loading Dye so the sample (12 μl maximum load) contains the suggested amount of DNA per band.

DNA sensitivity levels

The FlashGel™ System uses a proprietary stain that is 5–20 times more sensitive than ethidium bromide stain. In general, use per band DNA concentrations 1/5 of those normally used for ethidium bromide detection.

Optimal DNA load levels on FlashGel™ Recovery Cassettes are 50-500 ng per band. DNA levels may be adjusted to provide the best performance for the fragments of interest. High DNA load levels may not provide adequate resolution if the fragment of interest is relatively close in size to other contaminating fragments.

DNA Recovery

For optimal recovery efficiency (particularly of larger DNA fragments) add FlashGel™ Recovery Buffer to the Recovery wells prior to extracting DNA fragments.

To identify optimal conditions for recovered fragments use the FlashGel™ Control Fragment provided in the FlashGel™ Recovery Starter Pack.

Resolution of Recovered DNA

For best resolution of recovered DNA, dilute the sample to be run on a subsequent gel at least 1:1 with water (e.g. 2 μl recovered DNA, 2 μl water, 1 μl 5X FlashGel™ Loading Dye).

Gel Running Instructions

> ⚠ **CAUTION:** Wear gloves, lab coat and safety glasses when handling FlashGel™ Cassettes.

1. Prepare samples. Refer to Table 1 (pp 8-9) for recommended sample preparation.

2. Tear open the pouch and remove the cassette.

   NOTE: If cassette is wet, dry with a clean wipe.

   NOTE: Air pockets may be present between the gel and cassette. These will not affect band migration.

3. Place the cassette on a flat surface and pull the tab to remove the white well seal. Do not remove the clear seals covering the side vent holes.

4. Flood both tiers of wells with distilled or deionized water. Tilt the cassette and use a lint free wipe or small piece of blotting paper to remove excess fluid. Do not blot the wells directly.

   NOTE: For best results, flood wells with AccuGENE™ Molecular Biology Water (Cat. #51200).

> ⚠ **CAUTION:** To avoid accidental exposure to high voltage, do not flood wells while cassette is connected to the high voltage power supply.

5. Place the cassette on the dock and slide it into place; the cassette should snap securely into the dock. Slide the FlashGel™ Mask in place under the second tier of wells to minimize light passing through the wells and increase the ease of viewing as samples separate.

> ⚠ **CAUTION:** To avoid accidental exposure to high voltage, do not load samples while cassette is connected to the high voltage power supply.

> ⚠**CAUTION**: Use the FlashGel™ Mask to block light from the second tier of wells when using Recovery Cassettes.

6. Load samples and markers. Refer to Table 1 (pp 8-9) for sample preparation and recommended markers.
   NOTE: Use FlashGel™ Control Fragment to monitor recovery.

7. Connect the low voltage power supply to the dock by inserting the lead into the receptacle at the back of the dock and plugging in the power supply. This is the power supply to the light source.

8. Turn on the dock light by pressing the orange button on the top of the unit.

   NOTE: The light will automatically shut off after 10 minutes. Re-start by pressing the orange button.

> ⚠**CAUTION:** Handle or touch cassettes only after voltage is turned off and leads are disconnected.

9. Connect the high voltage leads to power supply and set power at recommended voltage (Table 1, pp 8-9).

   NOTE: Typical starting currents should range from 20-25 mA.

   NOTE: Some expression of buffer from the wells during run is normal. Wear gloves, lab coat and safety glasses when handling.

10. Allow run to proceed, monitoring migration of sample(s) to be recovered. **Just prior to desired sample(s) reaching recovery wells (2nd tier), turn off power and disconnect the high voltage cables**. Do not remove the cassette from the dock.

> ⚠**CAUTION:** Handle or touch cassettes only after voltage is turned off and leads are disconnected.

11. Blot excess buffer from the recovery well(s) and add 20 µl/well of FlashGel™ Recovery Buffer.

12. Remove FlashGel™ Mask, reconnect voltage cables, and restart power. Use FlashGel™ Visualization Glasses to observe DNA band migration.

13. Run until the band to be recovered has entered the recovery well, and the leading edge of the band has moved to the front edge of the recovery well.

    NOTE: When recovering more than one fragment from gel, recover smallest fragment first.

14. **Turn off power, disconnect leads**, and then use a pipette to remove the recovery buffer containing the DNA.

    > **CAUTION:** Wear gloves, lab coat and safety glasses when handling FlashGel™ Cassettes.

    NOTE: The amount recovered will not be the full 20 µl loaded into the well.

15. For recovery of large quantities of DNA (>350 ng), it may be necessary to repeat the electrophoresis-recovery cycle to retrieve the maximum DNA material.

    If necessary, add an additional 20 µl of FlashGel™ Recovery Buffer and start the run again until the sample has once again moved to the front edge of the recovery well, then recover. Repeat as necessary, being careful to always **turn off the power and disconnect the leads prior to recovering samples**.

    NOTE: The buffer capacity of the FlashGel™ Recovery Cassette will support ~12-14 minutes of total run time at 275 V. Use care in recovery of multiple fragments or exceptionally large fragments to avoid excess run time.

NOTE: It may be necessary to repeat steps 10 – 15 when recovering sheared DNA if your size selection is wider than the recovery well. Utilize the different cassette concentrations available to optimize your size selection needs.

16. Sample recoveries may be quickly assessed using the standard FlashGel™ Markers for DNA and/or the FlashGel™ QuantLadder.

    Prepare sample of recovered DNA by combining equal volumes of recovered DNA and water then adding appropriate amount of 5X FlashGel™ Loading Dye. The volume of DNA sample for recovery check depends upon the amount of input DNA loaded on the recovery gel. Estimate the amount of DNA in the recovered sample by comparison to bands in the FlashGel™ QuantLadder (Table 3, page 31; Fig. 8, page 32).

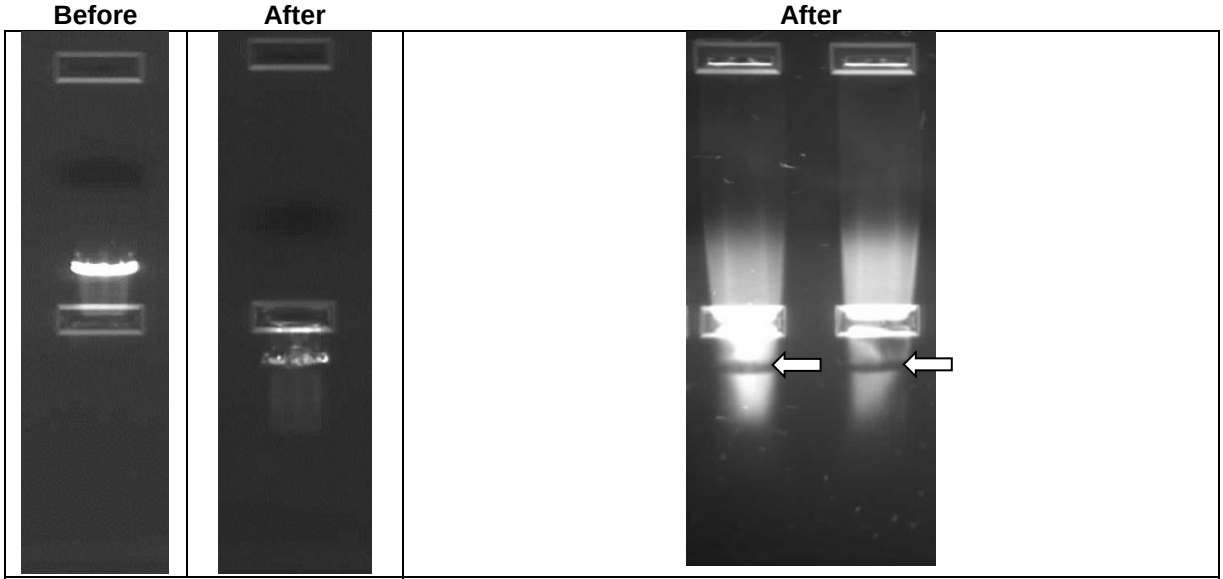
    NOTE: Depending upon the amount of run time used for the recovery step, the same FlashGel™ Cassette may be used to check the recovered sample. The cassette will be functional up to ~ 12-14 minutes of total run time at 275 V.

Reference Information

Table 3. FlashGel™ QuantLadder DNA Levels

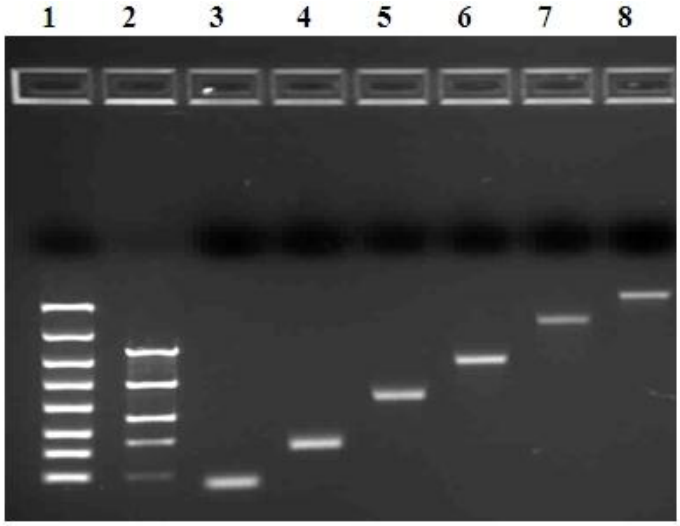
Fragment	2.5 µl Load	5.0 µl Load
1500 bp	15 ng	30 ng
800 bp	10.5 ng	21 ng
400 bp	7.5 ng	15 ng
250 bp	3.75 ng	7.5 ng
100 bp	1.5 ng	3 ng

Fig. 7. Images of FlashGel™ Recovery Cassettes Before and After Recovery

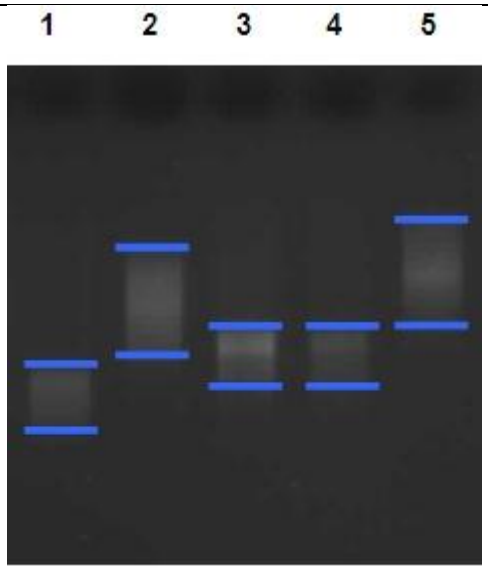


1000 bp DNA Fragment (300 ng load)	Samples from two lanes removed from a 2.2% FlashGel™ Recovery Cassette. Several seconds of 275V current applied after samples recovered show selection removed. Samples of higher molecular weight are now ready to be recovered.

Fig. 8. Recovery of Wide Size Range of DNA Fragments on FlashGel™ DNA Cassette



Samples (100 ng) of DNA fragments were separated and recovered using the FlashGel™ Recovery System. Image above shows the analysis of 3 µl aliquots of recovered DNA using a 1.2% DNA FlashGel™ Cassette.



Recovered samples of various size selections from fragmented genomic lambda DNA. Lanes contain DNA extractions from several experiments taken from 1.2% FlashGel™ Recovery Cassettes.

Sample Lanes:

Lane 1: FlashGel™ DNA Marker 100-4000 bp

Lane 2: FlashGel™ QuantLadder (2.5 µL)

Lane 3: 50 bp DNA fragment

Lane 4: 200 bp DNA fragment

Lane 5: 500 bp DNA fragment

Lane 6: 1000 bp DNA fragment

Lane 7: 2000 bp DNA fragment

Lane 8: 4000 bp DNA fragment

Sample Lanes:

Lane 1: 280 – 550 bp*

Lane 2: 650 – 1,190 bp*

Lane 3: 425 – 800 bp*

Lane 4: 425 – 800 bp*
(smaller load volume of lane 3)

Lane 5: 800 – 3,000 bp*

*Size ranges were determined by gel electrophoresis

Ordering Information

FlashGel™ System

Cat No.	Description	Size/Format
57025	FlashGel™ Dock	One size
57040	FlashGel™ Camera	One size
57068	FlashGel™ Power Supply	One size
57062	FlashGel™ Device Pack	Includes FlashGel™ Dock, FlashGel™ Power Supply, and FlashGel™ Camera
57069	FlashGel™ Power Supply Pack	Includes FlashGel™ Dock and FlashGel™ Power Supply
57065	FlashGel™ Camera Pack	Includes FlashGel™ Dock and FlashGel™ Camera

FlashGel™ System for DNA

Cat No.	Description	Size/Format
57023	FlashGel™ DNA Cassettes	1.2% agarose, 12+1 well single-tier format, 9pk
57029	FlashGel™ DNA Cassettes	1.2% agarose, 16+1 well double-tier format, 9pk
57031	FlashGel™ DNA Cassettes	2.2% agarose, 12+1 well single-tier format, 9pk
57032	FlashGel™ DNA Cassettes	2.2% agarose, 16+1 well double-tier format, 9pk
50462	FlashGel™ Loading Dye	5 x 1 ml vials 5X concentration
50473	FlashGel™ DNA Marker 100 bp – 4 kb	500 µl Ready-to-load *Recommended for 1.2% cassettes* Band Sizes: 100/200/300/500/800/1250/2000/4000 bp

FlashGel™ System for DNA (cont.)

Cat No.	Description	Size/Format
57033	FlashGel™ DNA Marker 50 bp – 1.5 kb	500 µl Ready-to-load *Recommended for 2.2% cassettes* Band Sizes: 50/100/150/200/300/500/800/1500 bp
57034	FlashGel™ DNA Marker 100 bp – 3 kb	500 µl Ready-to-load *Recommended for double-tier cassettes* Band Sizes: 100/300/500/800/1500/3000 bp
50475	FlashGel™ QuantLadder	250 µl Ready-to-load Band Sizes: 100/250/400/800/1500
57026	FlashGel™ DNA Kit	Includes FlashGel™ DNA Cassettes 1.2% 12+1 well single tier 9pk, FlashGel™ Loading Dye, and FlashGel™ Marker 100 bp – 4 kb

FlashGel™ System for RNA

Cat No.	Description	Size/Format
57027	FlashGel™ RNA Cassettes	1.2% agarose, 12+1 well single-tier format, 9pk
57028	FlashGel™ RNA Cassettes	1.2% agarose, 16+1 well double-tier format, 9pk
50571	Formaldehyde Sample Buffer	RNA denaturing sample buffer, contains bromophenol blue and xylene cyanol, 5 x 1 ml
50462	FlashGel™ Loading Dye	RNA native sample buffer 5 x 1 ml vials 5X concentration
50577	FlashGel™ RNA Marker	0.5 bp – 9 kb 50 µg (1 µg/ml)
51200	AccuGENE™ Molecular Biology Water (DNase/RNase free)	For flooding sample wells and diluting RNA, 1L

FlashGel™ System for RNA (cont.)

Cat No.	Description	Size/Format
57024	FlashGel™ RNA Kit	Includes FlashGel™ RNA Cassettes 1.2% 12+1 well single tier 9pk, RNA Marker, Formaldehyde Sample Buffer, and Molecular Biology Water

FlashGel™ System for Recovery

Cat No.	Description	Size/Format
57051	FlashGel™ Recovery Cassettes	1.2% agarose 8 + 1 double-tier format, 9pk
57022	FlashGel™ Recovery Cassettes	2.2% agarose 8 + 1 double-tier format, 9pk
57060	FlashGel™ Recovery Buffer	2 x 500 µl vials
57050	FlashGel™ Recovery Kit	Includes FlashGel™ Recovery Cassettes 1.2% 8+1 well double tier 9pk, FlashGel™ Recovery Buffer, FlashGel™ Loading Dye, FlashGel™ QuantLadder, and Visualization Glasses

Notes

Warranty and Liability

This product was produced utilizing the highest practical standards of materials, workmanship, and design. Lonza Rockland, Inc. warrants that the product has been tested and will meet or exceed published specifications. This warranty is valid only if the product has been operated and maintained according to the instructions provided.

Lonza Rockland, Inc. warrants this product to be free from defects in materials and workmanship under normal service for one year from date of shipment. If the product proves defective during this period, Lonza Rockland, Inc., will repair or replace it at our option, free of charge, if returned to us postage prepaid. This warranty does not cover: damage in transit, damage caused by carelessness, misuse or neglect, normal wear through frequent use, damage caused by solvent corrosion, damage caused by improper handling or user alteration, nor unsatisfactory performance as a result of conditions beyond our control. Lonza Rockland, Inc., shall in no event be liable for incidental nor consequential damages, including without limitation, lost profits, loss of income, loss of business opportunities, loss of use and other related damages, however caused, nor any damage arising from the incorrect use of the product.

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