ssDNA 100-R Kit

Care and Use Instructions

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ssDNA 100-R Kit

Introduction

The Beckman Coulter ssDNA 100-R Kit provides rapid separation and analysis of oligonucleotides having 10 to 100 bases of length. This kit features a replaceable gel buffer with a coated capillary for maximum reproducibility.

This kit is not recommended for use with a photodiode array detector (PDA).

This kit should be used with a UV Detector equipped with a 254 nm filter.

Kit Contents (Kit Reorder Number 477480)

Product Name	Quantity/Amount
DNA Capillary, 65 cm, 100 µm I.D.	2
ssDNA 100-R Gel	1.0 g (lyophilized)
Tris-Borate Buffer	1 bottle
7 M Urea	1 bottle
Test Mix pd(A) 40-60	0.2 O.D.
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Upon receipt:

- store the DNA Capillary and lyophilized gel at 2-8°C
- store Test Mix at -20°C
- store unreconstituted Tris-borate and urea buffer bottles at room temperature

The following items may be reordered separately from the Kit:

Product Name	Part Number
DNA Capillary	477477
ssDNA 100-R Gel	477621
pd(A) 40-60 Test Mix	477626
ssDNA 100 Buffer Kit (Tris-Borate, Urea)	338481

Materials Required but Not Provided

- Deionized Water
- Micropipet to deliver 500 µL
- Capillary Cartridge, blank, 100 x 200 µm aperture (PN 144738)
- PCR vials, 200 µL (PN 144709)
- Micro vial springs (PN 358821)
- 2 mL vials (PN 144980)
- 2 mL vial cap, red (PN 144648)
- PCR vial cap, gray (PN 144656)
- PCR vial holder (PN 144657)
- vortex mixer
- magnetic stir plate with stir bar
- 10 mL disposable syringes
- 0.2 µm membrane syringe filter
- 0.45 µm membrane syringe filter

Preparing the Tris-Borate-Urea Buffer

1. Add 135 mL of 16 to 18 megohm deionized water to the bottle containing the dry tris-borate buffer.
2. To dissolved the buffer, stir for 20 to 30 minutes using a stirring bar that has been cleaned with methanol and rinsed with deionized water. Be sure that the boric acid is completely dissolved before proceeding to the next step.
3. Slowly add the dry 7M urea to the bottle of dissolved tris-borate buffer while using the magnetic stir bar to mix the solution. The dissolution of urea is endothermic, so the bottle will get very cold.
   After approximately two hours of stirring, the buffer solution should be clear. The Tris-Borate-Urea buffer is now ready for use.

CAUTION Do not heat the buffer solution to speed the warming process. This will shorten the usable life of the buffer.

4. Remove the required volume for the day's use and filter through 0.2 µm filter. Store the remainder at 2°C to 8°C.

NOTE The usable life of the reconstituted buffer is 30 days when stored at 2°C to 8°C.

Some magnetic stirrers produce enough heat to degrade the urea. A small piece of corrugated cardboard can be used as an insulator between the buffer and the stirrer to minimize the heating.

Using the Refrigerated Tris-Borate-Urea Buffer

1. If the buffer solution was previously reconstituted and refrigerated, bring the entire container of buffer to ambient temperature before use while stirring slowly and continuously with a clean stirring bar.
2. Remove the required volume to be used for the day and filter through a 0.2 µm disposable syringe filter into a clean container.
3. Pipet 1.7 mL of Tris-Borate-Urea buffer into each of the required 2 mL vials.
4. Seal the vials with the red caps.
5. Sonicate for 5 minutes to degas the buffer solution.

The following points are important when using refrigerated buffer solution:

- For optimal migration time reproducibility, replace the vials of tris-borate-urea buffer after 18 runs.
- The current should be monitored at all times. Ionic strength changes, gel degradation, and/or the formation of bubbles are indicated by change in the average current value, or current fluctuations.
- Use only Beckman Coulter Tris-Borate buffer and 7M Urea with the Beckman Coulter DNA Capillary. The purity of the buffer raw material components is critical to the life of the coated capillary and of the buffer.
  Do not substitute buffer components from other vendors.

Preparing ssDNA 100-R Gel

1. Add 5.0 mL of filtered tris-borate-urea buffer to the lyophilized gel.
2. Use a clean magnetic stir bar to stir the solution until the gel is completely dissolved (4 to 6 hours).

For 1 to 8 runs:

1. Transfer 200 µL of the filtered gel into a PCR vial.
2. Centrifuge (6,000 rpm maximum) the vial for no more than 2 minutes to remove air bubbles.
3. Place the PCR vial in a vial holder equipped with a spring and seal it with a gray cap.

For Multiple Runs (more than 8):

1. Filter the gel through a 0.45 µm disposable syringe filter and pipet 1.7 mL into a 2 mL vial.
2. Seal the vial with a red cap and sonicate the vial 5 times for 30 seconds, each time.
3. Allow the air bubbles to rise to the surface between each sonicating cycle.

The following points are important when using ssDNA 100-R gel:

- On-board stability for a 2 mL vial filled with gel is 24 hours.
- If using 200 µL of gel, do not leave the gel in the sample tray for more than 5 hours. This may result in an increased migration time due to an increase in the viscosity of gel.
- For optimal migration time reproducibility, replace the gel in the capillary after every 8 runs.
- The shelf life of the reconstituted gel is 30 days, when reconstituted with freshly prepared buffer, and stored at 2°C to 8°C.

Installing the Coated Capillary

The recommended capillary length is 20 cm to the window; 30.2 cm total length. Before use on the P/ACE MDQ instrument, the DNA Capillary must be installed in a cartridge, according to the Capillary Replacement Procedure (PN 266910). This procedure and the required tools are included in the Capillary Cartridge Replacement Kit. Some modifications to the Capillary Replacement Procedure are necessary to make the procedure appropriate for installation of the DNA Capillary. The following note describes the necessary modifications to complete the building of a capillary cartridge without damaging the coating.

NOTE After trimming a capillary end, dehydration of the coating inside the capillary begins to occur after 10 minutes. This dry portion of the capillary becomes unusable. **Do not** cut the capillary to its final length before placing it in the MDQ cartridge. First, cut the end-caps at both sides and then install the capillary into the cartridge (as instructed in the Capillary Replacement Procedure). Before trimming the capillary ends, have two 2 mL vials filled with deionized water and sealed with red caps. As soon as the final trimming is done, submerged both ends inside the water-filled vials. Never let the capillary ends be exposed to air for no more than 3 minutes.

When not in use, always rinse and store the capillary in unused Gel Buffer at 2°C to 8°C with both capillary ends submerged in Tris-Borate-Urea buffer.

ssDNA 100-R Method

The ssDNA-100R Application kit requires two methods. The first method fills the capillary with ssDNA-100R gel, performs an equilibration step, injects the sample and separates it. The second method is shorter because it only injects the sample and separates it.

Always use the gel filling method first. After running the first method, the second method can be used up to 20 times, consecutively. However, if resolution is lost, perform the first method again.

Create both methods as shown in Figures 1-4. Both methods have the same Initial Conditions (Fig. 1) and Detector Settings (Fig. 2). The Time Program is different for each method. Fig. 3 shows the Time Program for the first method. After creating this method, save it as "ssDNA-100R gel filling.met". Fig. 4 shows the time program for the second method, which should be saved as "ssDNA-100R NO gel filling.met".

NOTE The gel inside the capillary may be replaced when needed (for example, when losing resolution). Follow the Gel Replacement and Capillary Equilibration Method each time the gel is replaced.

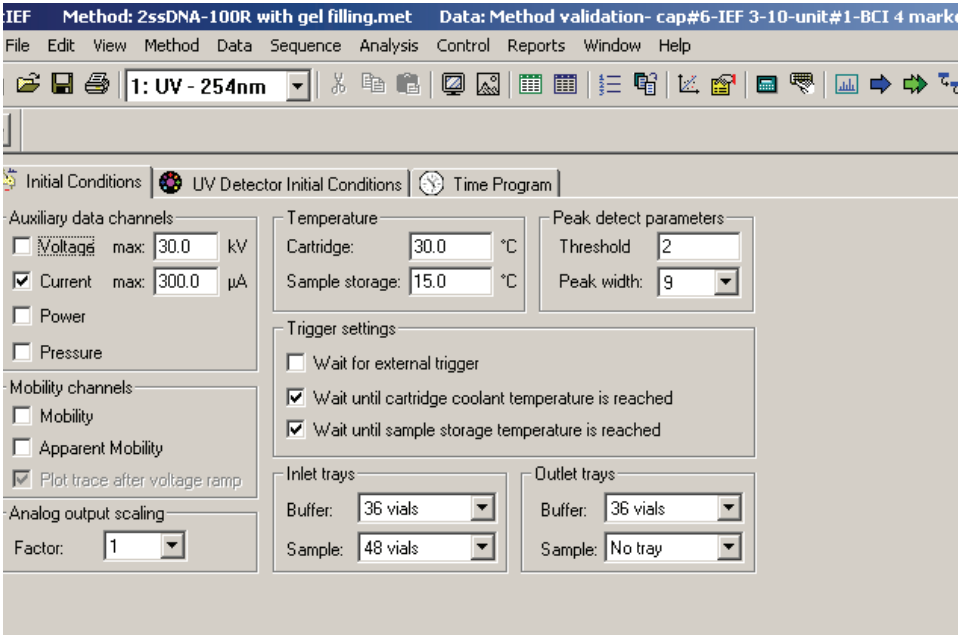

Figure 1 Initial conditions for both ssDNA 100-R methods.

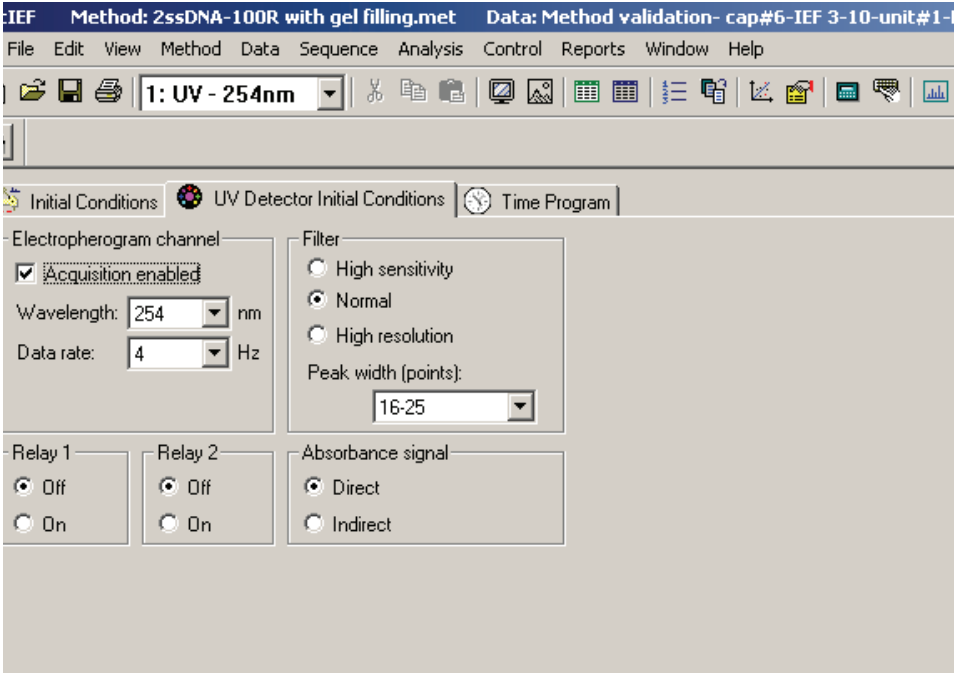

Figure 2 UV Detector settings for both ssDNA 100-R methods.

nditions | UV Detector Initial Conditions | Time Program

Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
	Rinse - Pressure	70.0 psi	5.00 min	BI:B1	BO:B1	forward	fill cap with ssDNA-100 gel into vial filled with w
	Inject - Voltage	1.0 KV	1.0 sec	BI:A2	BO:A2	No override, reverse polarity	cleaning tips w/water vials at both sides
	Inject - Voltage	1.0 KV	1.0 sec	BI:A3	BO:A3	No override, reverse polarity	cleaning tips w/water using another pair of vials
	Separate - Voltage	3.0 KV	2.00 min	BI:B2	BO:B2	0.17 Min ramp, reverse polarity	preelectrophoresis between buffer vials (do not
	Separate - Voltage	5.0 KV	2.00 min	BI:B2	BO:B2	0.17 Min ramp, reverse polarity	preelectrophoresis between buffer vials (do not
	Separate - Voltage	9.3 KV	10.00 min	BI:B2	BO:B2	0.17 Min ramp, reverse polarity	pre-electrophoresis between buffer vials (do not
	Inject - Voltage	3.0 KV	4.0 sec	BI:A4	BO:A4	No override, reverse polarity	cleaning tips with water using a different pair of
	Inject - Voltage	3.0 KV	4.0 sec	SI:A1	BO:B2	Override, reverse polarity	sample injection against buffer vial
)	Separate - Voltage	9.3 KV	40.00 min	BI:B2	BO:B2	0.17 Min ramp, reverse polarity	use buffer vials
)	Autozero						
)0	End						

Figure 3 Time Program for ssDNA 100-R method with gel filling and equilibration.

onditions | UV Detector Initial Conditions | Time Program

Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	
	Wait		0.00 min	BI:A1	BO:A1		H20 dip
	Inject - Voltage	3.0 KV	4.0 sec	SI:A1	BO:B2	Override, reverse polarity	sample inject
	Wait		0.00 min	BI:A1	BO:A1		H20 dip
10	Separate - Voltage	9.3 KV	40.00 min	BI:B2	BO:B2	0.17 Min ramp, reverse polarity	used buffer v
10	Autozero						
.00	End						

Figure 4 Time Program for ssDNA 100-R method without gel filling and equilibration.

Preparing the Test Mix

1. Add 500 µL of filtered deionized water (through 0.2 µm filter) to the vial labeled pd(A) 40-60 and mix well.
2. Pipette 100 µL of the test mix solution into a 200 µL PCR vial.
3. Place the test mix vial in a PCR holder equipped with a spring. Seal with a gray cap.
4. Transfer 100 µL aliquots of the remaining prepared test mix to sealable 400 µL vials and store frozen until needed.

CAUTION The lyophilized Test Mix has a shelf life of one year, when stored at -20°C. However, the reconstituted Test Mix deteriorates after several days at room temperature. Therefore, the reconstituted Test Mix should be stored frozen at -20°C, when not in use.

Preparing the Oligonucleotide Sample

The oligonucleotide sample concentration should be between 5 to 10 O.D./mL. Follow the same procedures as used in the preparation of the test mix, as noted above.

Performing a Test Run

1. Prepare six 2 mL vials filled with deionized water and place them as shown.
2. Fill a vial half way with deionized water and place it at the Waste position.
3. Place the 2 Tris-Borate-Urea buffer vials as shown above.
4. Place the ssDNA 100-R gel vial in position B1, in the inlet tray.
5. Place the Sample in S1 (not shown above) in the Sample Tray.

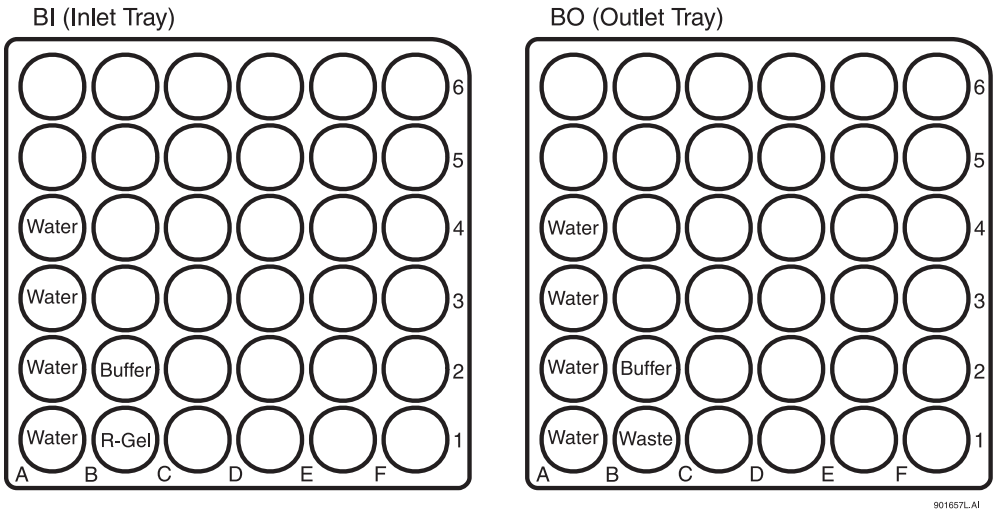

Figure 5 100-R Gel Tray configuration.

Important Notes

- The DNA Coated Capillary is filled with a storage solution which must be replaced with ssDNA 100-R Gel, for first time use. This is done by performing a 20 minute rinse with the ssDNA 100-R gel.
- Once the Capillary has been filled with the gel and equilibrated, separation can be carried out. It is not necessary to replace the gel after each run. However, the gel can be replaced if needed, i.e. when losing resolution, by using the ssDNA 100-R Filling and Equilibration method.
- The capillary supplied in this kit is 65 cm long. For this application, we recommend a 20 cm length from the capillary injection site to the window with the total length of 30.2. If needed, use a longer capillary and the same field strength (volts/cm), to optimize the separation.

Checking the Results

The pd(A) 40-60 Test Mix as used should give a baseline separation of all the oligonucleotides in 35 minutes (when using a 30.2 cm capillary at a field strength of 300 V/cm) as shown in Figure 6. Peak intensity may vary due to batch-to-batch variation. Refer to the electropherogram provided with the test mix to check your results. The current should remain fairly stable and run between 6 and 8 µA.

The high resolving power of this kit can achieve not only single-base resolution of each oligonucleotide, but also show the appearance of shoulders on the major peaks which may be the dephosphorylated form of each oligonucleotide or other contamination. However, the intensity of these shoulders may vary due to the lot-to-lot variation in test mix preparation.

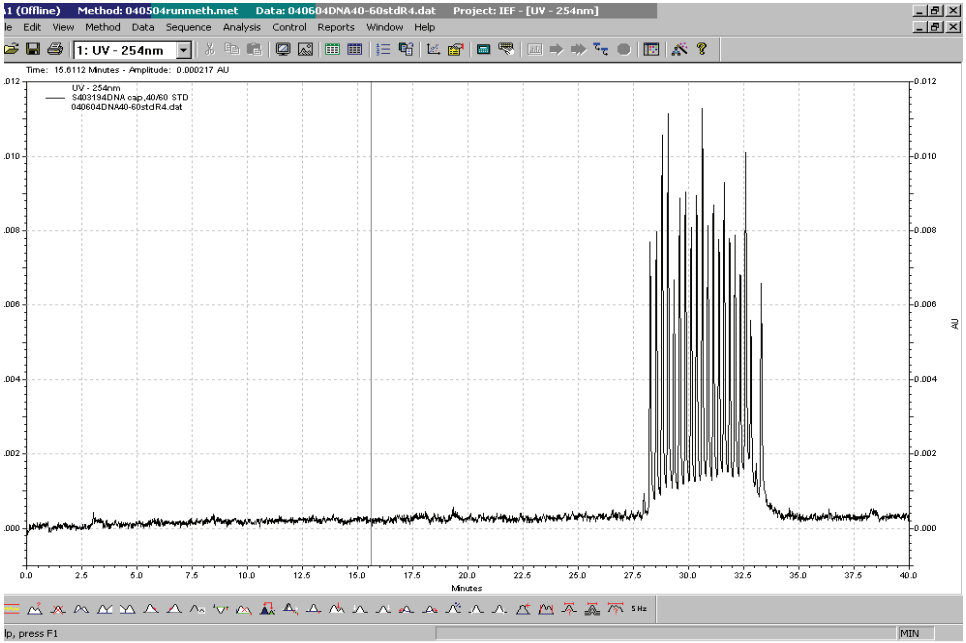

Figure 6 Electropherogram of pd(A) 40-60 Test Mix.

Troubleshooting Guide

Problem	Possible Cause	Corrective Action
Reduced resolution	Deteriorating buffer or test mix.	Change to a new buffer or test mix.
	Deteriorating gel inside the capillary.	Replace the gel and repeat the equilibration procedure.
	Bad Capillary coating	Replace the DNA capillary.
Low or unsteady current	Deteriorating buffer.	Replace the buffer.
	Capillary plugged.	Replace the gel and repeat the equilibration procedure.
	Air bubbled in the gel.	Replace the gel and repeat the equilibration procedure.
	Broken capillary.	Replace the capillary.
Changing migration time from run to run	Capillary not equilibrated	Run equilibration method.
	Deteriorating gel.	Replace the gel.
	Contamination on the electrodes.	Clean the electrodes, capillary ends.
No Peaks or low absorbance	Plugged or dried capillary.	Replace the gel and repeat the equilibration procedure.
	Broken capillary.	Replace the capillary.
	Inadequate capillary equilibration.	Replace the gel and repeat the equilibration procedure.
Spikes	Air in the Gel Buffer.	Make sure that the buffer is at room temperature and that the air is removed.
	Contamination or microparticles in gel or buffer.	Replace the gel and buffer vials with freshly filtered solutions.
Unstable or shifting baseline	Inadequate capillary equilibration.	Replace the gel and repeat the equilibration procedure.
	Degraded ssDNA 100-R gel	Replace with new gel.
	Degraded Tris-Borate-Urea buffer	Replace with fresh buffer.

Additional Information

Other Beckman Coulter CE Chemistry Kits:

Subject	Product & Part Number
DNA	
For analysis of double-stranded nucleic acids by UV detection:	dsDNA 1000 Kit (PN 477410) dsDNA 20,000 Kit (PN 477475)
For analysis of double-stranded Nucleic acids by LIF detection:	EnhanCE (PN 477409), for use with the above mentioned kits
Small Molecules	
For Chiral Separations:	Chiral Methods Development Kit (PN 477450)
For separation of small molecules on positively charged capillary:	Amine Methods Development Kit/Small Molecules (PN 477430)
Proteins	
For analysis that requires minimal EOF and a neutral capillary surface:	Protein Methods Development Chemistries (PN 477445)
For protein molecular weight and purity determinations:	ProteomeLab™ SDS-Gel HW Analysis Chemistries (PN 390953)
For determination of isoelectric points:	Isoelectric Focusing Chemistries (PN 477490)
For the analysis of sugars & glycoproteins:	Carbohydrate Labeling & Analysis Chemistries (PN 477600)
For the determination of IgG Purity:	ProteomeLab IgG Purity Heterogeneity Assay (PN A10663)

Other Beckman Coulter Literature

Separation of DNA by Capillary Electrophoresis (PN 607397)

Nucleic Acid Chemistry Kits for P/ACE Capillary Electrophoresis (BR-7936A)

Additional Technical Support

North America:

Contact Beckman Coulter Technical Support @ 800-742-2345.

Worldwide:

Contact your local Beckman Coulter Technical Support Associate.