

*Protocol for dsRNA Duplex Analysis using
Native Oligel[®]*



Introduction

Native Oligel[®] Gel has been designed to effectively separate single stranded oligos from duplex oligos. With no denaturant in solution, the gel can effectively separate oligo over a wide base range. An example separation is shown in Figure 1. Salts present in oligo preparation may affect injection efficiency. For best results, it is recommended that samples be diluted in water.

The gel and buffer are premixed and ready to be used, reducing hands on time and inconsistencies related to gel and buffer preparation.

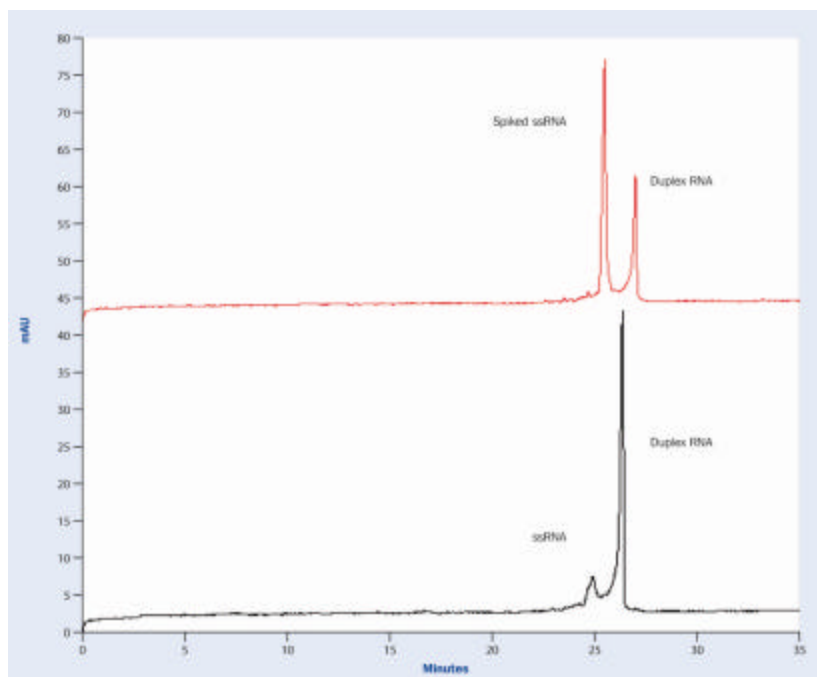


Figure 1: Electropherogram obtained from a mixture of oligonucleotides using *Native Oligel*[®] matrix.

Capillary Dimensions

The procedures are based on the use of a bare fused silica capillary with 100 μm i.d., 50 cm effective/60 cm total length.

Reagents needed

Part #	Description
scDN-475-0050	Capillary Conditioning Solution
scDN-515-0006	<i>Native Oligel</i> [®] Gel
scDN-565-0100	<i>Native Oligel</i> [®] Buffer
Optional:	
DN-500-0001	Native Oligel OQ Standard 1X Soln

Procedure

Instrument set up

1. Install the capillary as directed by instrument instruction manual.
2. Set cartridge temperature at 20°C.
3. Set detection wavelength at 254nm.

A. Capillary preconditioning, gel filling and pre run

1. Dispense 1.5mls Capillary conditioning solution (scDN-475-0050) into a glass vial and place in inlet tray.
2. Place a glass vial in the outlet tray to collect waste.
3. Program system to rinse new capillary with Capillary conditioning solution (scDN -475-0050) at 60 psi for 15 min.
4. Place 0.2mls *Native Oligel*[®] Gel (scDN-515-0006) into a PCR tube and centrifuge (6,000 rpm for 2 minutes) to remove air bubbles.
5. Place PCR tube into sample vial holder as directed by instrument instruction manual. Place vial in outlet tray.
6. Fill a glass vial with 1.5mls deionized water and place in inlet tray to collect waste from gel pumping.
7. Program system to fill capillary at 100 psi for 240 minutes.
8. After capillary is filled, perform pre-run at -9 kV for 60 min to stabilize current.

B. Sample analysis

1. Fill 2 glass vials with 1.5mls *Native Oligel*[®] Buffer (scDN -565-0100). Place one vial in the inlet tray and one vial in the outlet tray.
2. Place 50µl of the OQ standard (DN-500-0001) into a PCR tube. Place the PCR tube into a sample vial holder as directed by instrument instruction manual.
3. Place samples in individual vials, using PCR tubes if necessary.
4. Fill a glass vial with deionized water to perform a water dip prior to sample injection (inlet tray).
5. Complete water dip for 30 seconds.
6. Perform sample injection at -5 kV for 3-10 seconds. Injection time is dependent on sample concentration and salt content. *Inject OQ standard at -5kV for 3 seconds.*
7. Separatate samples at -9kV for 45 minutes.
8. Detection wavelength: 254 nm.
9. Repeat step 5-9 for subsequent sample analysis.

C. Gel filling and pre run

1. Daily place 0.2mls *Native Oligel*[®] Gel (scDN-515-0006) into a PCR tube and centrifuge (6,000 rpm for 2 minutes) to remove air bubbles.
2. Place PCR tube into sample vial holder as directed by instrument instruction manual. Place vial in outlet tray.
3. Fill a glass vial with 1.5mls deionized water and place in inlet tray to collect waste from gel pumping.
4. Program system to fill capillary at 100 psi for 60 minutes.
5. After capillary is filled, perform pre-run at -9 kV for 60 min to stabilize current.

If separation resolution/performance decreases to an unacceptable level, repeat from Step C1-C5 to refresh gel matrix in the capillary.

If refreshing gel matrix does not restore the separation performance, repeat from Step A1-A8 to re-condition and refill the capillary.

Shutdown and clean up

1. If samples will be run within 1 day of last use, it is recommended that a shutdown method be established and run. Consult instrument owners manual for appropriate shutdown sequences.
2. If no additional sample will be run, it is recommended that a clean up method be established and run. Consult instrument owners manual for appropriate clean up method.

*procedure tested on Beckman MDQ

Notes

1. Typical sample concentration is 1 μM diluted in deionized water. The injection time and voltage can be adjusted to yield a reasonable peak height without a loss in separation resolution.
2. Any anionic salts present in the sample will reduce the injection efficiency and require an increase in injection voltage and/or time.
3. A voltage pre-run is required each time the capillary is flushed with fresh gel matrix.
4. The reagents are made with room temperature stable polymers; it is recommended, however that unused reagents at 2-8°C for optimum performance.

For technical support – contact tech-support-CE@aati-us.com or phone 515-294-1690