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CEQ™ 8000

Genetic Analysis System

Labeling and Purification of Oligonucleotides with the Beckman Coulter, Inc. WellRED Dye-labeled Phosphoramidites

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Customer Support

Please address all questions related to this product to DLPAhelp@beckman.com. Include your name, address and phone number in the body of your email.

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Labeling and Purification of Oligonucleotides with the Beckman Coulter, Inc. WellRED Dye-labeled Phosphoramidites

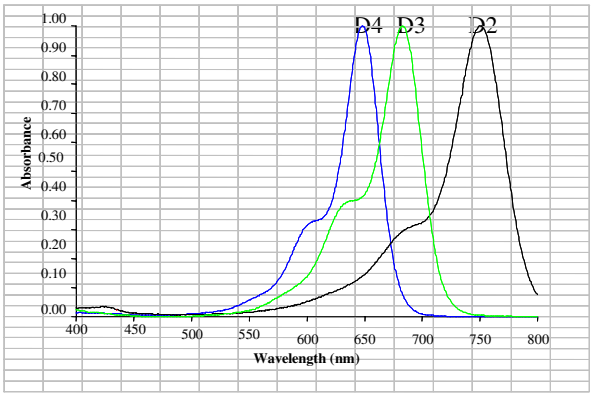
The WellRED Dye-labeled Phosphoramidites are cyanine-based fluorescent dyes with high extinction coefficients that absorb in the near infrared spectral region. These dyes were designed specifically for use with the CEQ™ series Genetic Analysis Systems, and are excited to fluoresce using diode lasers; much more stable and cost effective than traditional argon ion lasers.

The WellRED Dye-labeled Phosphoramidites are easily coupled to the 5' end of oligonucleotides using commercial DNA synthesizers (ABI 392, ABI 394, and Expedite). The resulting labeled oligonucleotides may be used for direct hybridization or in PCR amplification processes. Labeled DNA fragments may be detected, quantitated and sized by the CEQ™ 8000 Genetic Analysis System.

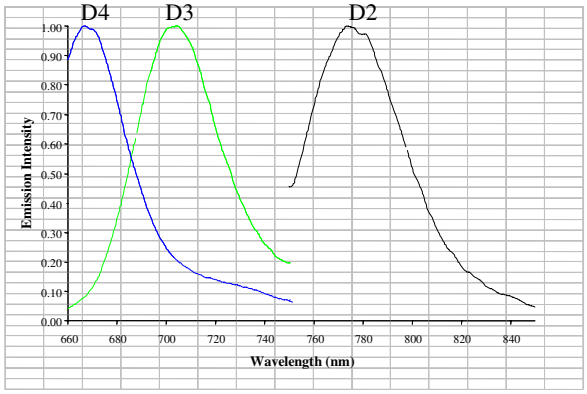
Absorbance and Emission spectral data:

Spectral Data		
Dye	**Absorbance maximum**	**Emission maximum**
D2-PA	750 nm	770 nm
D3-PA	685 nm	706 nm
D4-PA	650 nm	670 nm
Physical data		
Dye	**Phosphoramidite MW**	**Dilution**
D2-PA	829.95	100 mg/mL
D3-PA	863.97	100 mg/mL
D4-PA	763.84	100 mg/mL

Absorbance Spectra of Dyes



Emission Spectra of Dyes



Section I Protocol for Labeling Oligonucleotides with Dye-phosphoramidite on the ABI 392* or ABI 394* DNA/RNA Synthesizer

*Please refer to the instrument's User's Manual for detailed operating instructions.

Required Reagents:

Description	Part#	Quantity
D2-PA	608147	100mg
D3-PA	608146	100mg
D4-PA	608145	100mg
Pac-dA-CE phosphoramidite	Glen Research # 10-1601-xx	as needed
iPr-Pac-dG-CE phosphoramidite	Glen Research # 10-1621-xx	as needed
Ac-dC phosphoramidite	Glen Research # 10-1015-xx	as needed
Standard T phosphoramidite	Glen Research # 10-1030-xx	as needed
Anhydrous Acetonitrile (ACN)	EM Science # AX0151-1	1mL
0.05M Potassium Carbonate/Methanol	Glen Research # 60-4600-30	1mL
Ammonia Hydroxide	J. T. Baker # 9733-01	1mL
HPLC water (no DEPC)		250µL

Required Equipment and Materials:

Description	Quantity
Microcentrifuge tube	as needed
Pipettes: Eppendorf 0.5-10µL, 100-1000µL	1 each
ABI 392 or 394 DNA synthesizer	as needed
Savant Speed-vac	as needed

BCI Dye-labeled Phosphoramidites (D2-PA, D3-PA, and D4-PA phosphoramidites) should be stored in the dark at -20°C, or below, in a non frost-free freezer.

Protocol

1) Determine scale of synthesis and synthesize the desired oligonucleotide sequence using "pac Ultramild amidites" through the DMT On of the final base. It is important to use the "pac Ultramild amidites" so that mild cleave and deprotection conditions can be used.

To achieve the highest labeling efficiency it is recommended that the oligonucleotides to be labeled be "batched" for labeling. To do batch processing, synthesize all of the oligonucleotides through the final base with the DMT On, then reconstitute the appropriate DLPA and finish the labeling process.

2) Allow the vial containing the dye-labeled Phosphoramidite (DLPA) to come to room temperature before use (approximately 10 minutes). This is to avoid moisture condensation that could damage the dye.

3) Dissolve 100mg of the appropriate DLPA in 1mL anhydrous ACN. The DLPAs should be dissolved in super anhydrous ACN for 10 minutes at room temperature before installation on the DNA synthesizer.

4) The DNA synthesizer instrument (ABI 392 or 394) should be programmed for a 10 minute coupling period.

5) Cleavage and deprotection of the oligonucleotide is performed by using NH_4OH for 4 hours at room temperature in the case of D4-PA and D3-PA, and by using 0.05M K_2CO_3/MeOH for 8-16 hours at room temperature in the case of D2-PA.

The cleavage and deprotection is performed most efficiently by attaching the column to two syringes and mixing by pushing the solution back and forth between the syringes several times every 20-30 minutes. Store the syringe-column in the dark between mixing steps.

6) After deprotection, transfer the primer/reagent mixture to a microcentrifuge tube and evaporate the reagent until completely dry in a vacuum dryer **without heat**. Dissolve the primer in 300µL of HPLC Water (no DEPC), mix by vortexing, and spin briefly in a microcentrifuge. Evaporate the primer again until completely dry by placing in a vacuum dryer **without heat**.

Use of heat during the drying steps may cause decomposition of the dye.

7) At this point choose which method of purification, Reverse Phase Cartridge or HPLC, that will be used and proceed to the appropriate protocol for further instructions.

Section II Protocol for Labeling Oligonucleotides with Dye-phosphoramidite on the Expedite DNA/RNA Synthesizer*

*Please refer to the instrument's User's Manual for detailed operating instructions.

Required Reagents:

Description	Part#	Quantity
D2-PA	608147	100mg
D3-PA	608146	100mg
D4-PA	608145	100mg
Pac-dA-CE phosphoramidite	Glen Research # 10-1601-xx	as needed
iPr-Pac-dG-CE phosphoramidite	Glen Research # 10-1621-xx	as needed
Ac-dC phosphoramidite	Glen Research # 10-1015-xx	as needed
Standard T phosphoramidite	Glen Research # 10-1030-xx	as needed
Anhydrous Acetonitrile (ACN)	EM Science # AX0151-1	1mL
0.05M Potassium Carbonate/Methanol	Glen Research # 60-4600-30	1mL
Ammonia Hydroxide	J. T. Baker # 9733-01	1mL
HPLC water (no DEPC)		250µL

Required Equipment and Materials:

Description	Quantity
Microcentrifuge tube	as needed
Pipettes: Eppendorf 0.5-10µL, 100-1000µL	1 each
Expedite DNA synthesizer	as needed
Savant Speed-vac	as needed

BCI Dye-labeled Phosphoramidites (D2-PA, D3-PA, and D4-PA phosphoramidites) should be stored in the dark at -20°C in a non frost-free freezer.

Protocol

1) Determine the scale of the synthesis and synthesize the desired oligonucleotide sequence using "pac Ultramild amidites" through the DMT On of the final base. It is important to use the "pac Ultramild amidites" so that mild cleave and deprotection conditions can be used.

To achieve the highest labeling efficiency it is recommended that the oligonucleotides to be labeled be "batched" for labeling. To do batch processing, synthesize all of the oligonucleotides through the final base with the DMT On, then reconstitute the appropriate DLPA and finish the labeling process.

2) Allow the vial containing the dye-labeled Phosphoramidite (DLPA) to come to room temperature before use (approximately 10 minutes). This is to avoid moisture condensation that could damage the dye.

3) Dissolve 100mg of the appropriate (DLPA) in 2mL anhydrous ACN. DLPAs should be dissolved in super anhydrous ACN for 10 minutes at room temperature before installation on the DNA synthesizer.

4) The Expedite DNA synthesizer instrument should be programmed to couple DLPA (from position 5, 6 or 7) as follows:

Function	Mode	Amount/Argl	Time (sec) /Arg2	Description
Deblocking				
144 /* Index Fract. Coll.	*/ NA	1	0	"Event out ON"
0 /* Default	*/ WAIT	0	1.5	"WAIT"
141 /* Trityl Mon. On/Off	*/ NA	1	1	"START data collection"
16 /* Dblk	*/ PULSE	10	0	"Dblk to column"
16 /* Dblk	*/ PULSE	50	49	"Deblock"
38 /* Diverted Wsh A	*/ PULSE	40	0	"Flush system with Wsh A"
141 /* Trityl Mon. On/Off	*/ NA	0	1	"STOP data collection"
144 /* Index Fract. Coll.	*/ NA	2	0	"Event out OFF"
Coupling				
1 /* Wsh	*/ PULSE	5	0	"Flush system with Wsh"
2 /* Act	*/ PULSE	5	0	"Flush system with Act"
22 /* 5 + Act	*/ PULSE	10	0	"Monomer + Act to column"
2 /* Act	*/ PULSE	1	0	"Chase with Act"
2 /* Act	*/ PULSE	4	600	"10min Couple monomer"
1 /* Wsh	*/ PULSE	2	31	"Couple monomer"
1 /* Wsh	*/ PULSE	30	0	"Flush system with Wsh"
Oxidizing				
15 /* OX	*/ PULSE	15	0	"Ox to column"
12 /* Wsh A	*/ PULSE	15	0	"Flush system with Wsh A"
Capping				
12 /* Wsh A	*/ PULSE	30	0	"End of cycle wash"

1) Note that the final Capping process is omitted for the DLPA addition (No Cap A or Cap B steps) in the above program.

2) Cleavage and deprotection of the oligonucleotide is performed by using NH_4OH for 6 to 16 hours at room temperature in the case of D4-PA and D3-PA, and by using 0.05M K_2CO_3/MeOH for 8-16 hours at room temperature in the case of D2-PA.

The cleavage and deprotection is performed most efficiently by attaching the column to two syringes and mixing by pushing the solution back and forth between the syringes several times every 20-30 minutes. Store the syringe-column in the dark between mixing steps.

3) After deprotection, transfer the primer/reagent mixture to a microcentrifuge tube and evaporate the reagent until completely dry in a vacuum dryer **without heat**. Dissolve the primer in 300µL of HPLC Water (no DEPC), mix by vortexing, and spin briefly in a microcentrifuge. Evaporate the primer again until completely dry by placing in a vacuum dryer **without heat**.

Use of heat during the drying steps will cause decomposition of the dye.

4) At this point choose which method of purification, Reverse Phase Cartridge or HPLC, that will be used and proceed to the appropriate protocol for further instructions.

Section III Protocol for the purification of Dye-labeled Oligonucleotides by Reverse Phase Cartridge

Required Reagents:

Description	Part#	Quantity
Anhydrous Acetonitrile (ACN) HPLC grade	EM Science # AX0151-1	5mL
2M Triethylammonium acetate (TEAA) buffer, pH 7	Glen Research 60-4110-57	5mL
0.1M TEAA buffer, pH 7		1mL
10% ACN in 0.1M TEAA pH 7		15mL
50% ACN in HPLC water		1mL
HPLC water (no DEPC)		10mL

Required Equipment and Materials:

Description	Part#	Quantity
Poly-Pak™ cartridge	Glen Research, # 60-1100-10	1
10 mL Disposable Syringe	Becton Dickinson	1
Microcentrifuge tube		as needed
Pipettes: 0.5-10µL, 100-1000µL		1 each
Savant Speed-vac		as needed

Poly-Pak™ cartridges are used for purification of oligonucleotides produced on a 50 nmole or 200 nmole scale.

Protocol

Cartridge Assembly:

1) Connect a syringe (10mL disposable syringe works well) to the female luer of the cartridge and have the male luer terminate into waste.
2) Make certain that all fittings are tight and secure.

Remove the whole syringe from the cartridge before removing the plunger. The cartridge can be immobilized using a laboratory clamp.

Cartridge Pre-load Preparation:

3) Pass 5mL of ACN (HPLC grade) through the cartridge to waste.
4) Pass 5mL of 2M TEAA (Triethylammonium acetate buffer, pH 7) through the cartridge to waste.

Sample Pre-load preparation:

5) Completely dry the dye-labeled oligonucleotide (after cleaving and deprotection with the appropriate reagent).
6) Dissolve the primer residue in 1mL 0.1M TEAA pH 7 buffer.

Sample Loading:

7) Pass the dissolved primer from step 6 through the cartridge at a rate of about 1 drop per second and collect the eluate.

8) Pass the eluate from step 7 through the cartridge a second time in the same manner.

Cartridge Washing:

9) Wash the cartridge 3 times with 5mL of 10% acetonitrile in 0.1M TEAA buffer pH 7.

10) Wash the cartridge with 10mL of deionized water.

Sample Elution:

11) Slowly pass, drop-by-drop, 1mL of 50% of acetonitrile/deionized water (v/v) through the cartridge into a microcentrifuge tube.

12) Evaporate the purified sample solution until completely dry in a vacuum **without heat**.

Use of heat during the drying steps will cause decomposition of the dye.

13) Store the dry sample in the dark at -20°C until use.

Section IV Protocol for the purification of Dye-labeled Oligonucleotides by Reverse Phase HPLC*

*Please refer to the instrument's User's Manual for detailed operating instructions.

Required Reagents:

Description	Part#	Quantity
2M Triethylammonium acetate (TEAA) buffer, pH 7	Glen Research 60-4110-57	as needed
0.05M Triethylammonium acetate (TEAA) buffer pH 7		0.5mL
Buffer A: 100% Acetonitrile	EM Science # AX0151-1	as needed
Buffer B: 50mM Ammonium Acetate buffer (pH 7.0)		as needed
Buffer C: 0.05M Triethylammonium acetate buffer, pH 10 (TEAA)		as needed
HPLC water (no DEPC)		as needed

Required Equipment and Materials:

Description	Part#	Quantity
HPLC		1
C18 HPLC column	Hamilton # 79422 (PRP-1, 7µm, 250x4.1mm)	1 each
Microcentrifuge tube		as needed
15mL conical tube		as needed
Pipettes: 0.5-10µL, 100-1000µL		1 each
Savant Speed-vac	model 210A	as needed

Protocol

1) Resuspend the dried primer in 100µL of 0.05M TEAA pH 7 and pipette the solution into an appropriate HPLC cartridge or tube.
2) Enter the appropriate sample parameters and dye separation method, from the chart below, into the HPLC software.
3) Purify the labeled oligonucleotide using the Hamilton PRP-1 Analytical HPLC column and separation buffers A, B, and C.

Flow rate: 1.5mL/minute for all methods

D2-PA

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	1.500	2.0	0.0	98.0	0.0	6
2	0.50	1.500	2.0	0.0	98.0	0.0	6
3	6.00	1.500	27.5	0.0	72.5	0.0	6
4	27.20	1.500	34.5	0.0	65.5	0.0	6
5	28.00	1.500	100.0	0.0	0.0	0.0	4
6	29.00	1.500	0.0	100.0	0.0	0.0	6
7	30.00	1.500	2.0	0.0	98.0	0.0	8
8	34.00	0.000	2.0	0.0	98.0	0.0	11

D3-PA

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	1.500	2.0	0.0	98.0	0.0	6
2	0.50	1.500	2.0	0.0	98.0	0.0	6
3	6.00	1.500	28.0	0.0	72.0	0.0	6
4	27.20	1.500	36.0	0.0	64.0	0.0	6
5	28.00	1.500	100.0	0.0	0.0	0.0	4
6	29.00	1.500	0.0	100.0	0.0	0.0	6
7	30.00	1.500	2.0	0.0	98.0	0.0	8
8	34.00	0.000	2.0	0.0	98.0	0.0	11

D4-PA

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	1.500	2.0	0.0	98.0	0.0	6
2	0.50	1.500	2.0	0.0	98.0	0.0	6
3	6.00	1.500	21.5	0.0	78.5	0.0	6
4	27.20	1.500	29.5	0.0	70.5	0.0	6
5	28.00	1.500	100.0	0.0	0.0	0.0	4
6	29.00	1.500	0.0	100.0	0.0	0.0	6
7	30.00	1.500	2.0	0.0	98.0	0.0	8
8	34.00	0.000	2.0	0.0	98.0	0.0	11

4) Combine the labeled primer peak fractions into one 15mL conical tube then completely dry the eluate in a vacuum dryer **without heat**. An example of an HPLC chromatogram with the labeled primer peak indicated is given below in figure 1.

Use of heat during the drying steps will cause decomposition of the dye.

5) Resuspend the purified primer in 250µL of HPLC water (no DEPC), transfer to a microcentrifuge tube and then evaporate until completely dry in a vacuum dryer **without heat**.

6) Repeat step 4.

7) Store the dry sample in the dark at -20°C until use.

Figure 1 HPLC Elution Chromatogram