LINK

Product Update

May 2016

Fluorescence Quenching

BlackBerry® and Dabsyl Quenchers

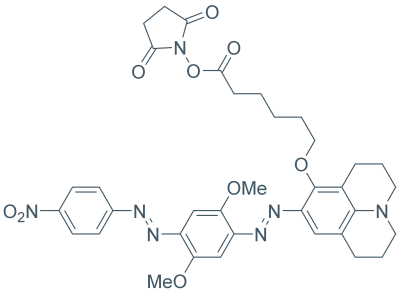
In response to customer requests for a wider range of quenchers, LINK has introduced three BlackBerry Quencher (BBQ®) 650 products - BBQ-650®-(DMT)-CE-Phosphoramidite (**2550**), 3'-BBQ-650® CPG II (**2427**), and BBQ-650® *N*-hydroxysuccinimide ester (**1378**), in addition to 3'-Dabsyl (**2426**).

We have introduced the BBQ®-650 products to cover all potential applications of this quencher:

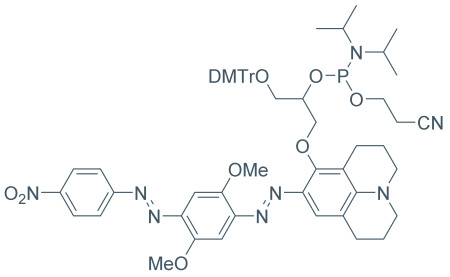
- The CPG **2426** to enable incorporation at the 3'-end of an oligonucleotide for use in *e.g.* Taqman® probes or Molecular Beacons®.
- The multiaddition amidite **2550** to enable incorporation anywhere in the oligonucleotide, *i.e.* 5', 3' or internally within the sequence. For instance for use in Scorpion® primers or Sunrise Probes (internally) or hybridisation probes (5' or 3').
- The NHS ester **1378** to enable conjugation to:
    - Oligonucleotides (post synthetically or on-column)
    - PNA oligos (N-terminus *via* an AEEA linker; 5005 or the C-terminus *via* lysine)

Using BBQ®-650 in conjunction with DDQ-1 (**2349**), dabcyl (**2085**, **2144** or **2374**) or dabsyl (**2426**) provides end users with an alternative to BHQ-1 / BHQ-2 combination.

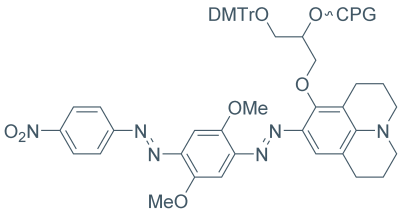
BBQ®-650 quenchers have a λ_{max} of 650nm with a quenching range from 550-750nm, therefore are useful when paired with cyanine or rhodamine dyes (see Figure 1 and Table 1 below). Whereas, DDQ-1, dabcyl and dabsyl have a quenching range of 400-550nm and are paired with fluorescein dyes such as FAM and TET.



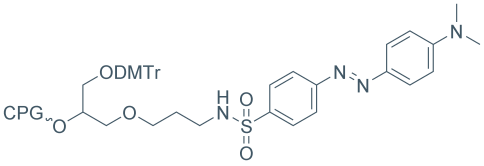
1378



2550



2427



2426

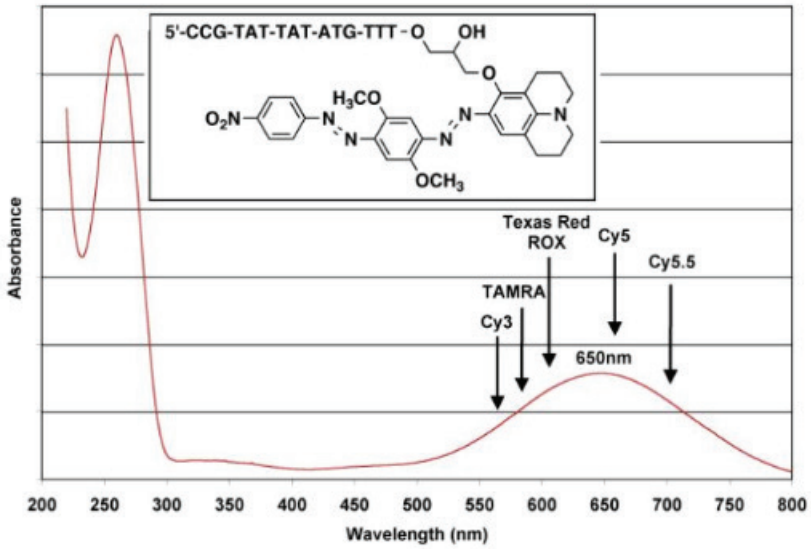


Figure 1. UV spectrum of 15mer oligonucleotide modified with BBQ® 650 CPG II (**2427**).

Table 1. Dye/Quencher Selection Chart. Note the dyes are listed in order of emission maxima; the colour scale is used only as a pictorial representation. Dyes and quenchers in bold are available from LINK.

Fluorophore	Abs. Max. (nm)	Em. Max. (nm)	Quencher
Cy5.5™	675	694	BBQ® 650 (550-750 Abs. Max. 650nm)
Alexa 647	650	668	BBQ® 650
Quasar® 670/Cyanine-5 (Cy5™)	647	667	BBQ® 650
BODIPY® 650/665-X	646	660	BBQ® 650
Pulsar® 650	460	650	BBQ® 650
BODIPY® 630/650-X	625	640	BBQ® 650
CAL Fluor® Red 635	618	637	BBQ® 650
BODIPY® TR-X/Alexa 594	590	617	BBQ® 650
CAL Fluor® Red 610	590	610	BBQ® 650
Texas Red X/Alexa 568	578	603	BBQ® 650
ROX	575	602	BBQ® 650 / BHQ®-2 (559-670 Abs. Max. 579nm)
Cy3.5™	581	596	BBQ® 650 / BHQ®-2
Redmond Red™	580	594	BBQ® 650 / BHQ®-2
BODIPY® 581/591	581	591	BBQ® 650 / BHQ®-2
CAL Fluor® Red 590	569	591	BBQ® 650 / BHQ®-2
TAMRA/Rhodamine Red-X	555	580	BBQ® 650 / BHQ®-2
Alexa 546	556	573	BBQ® 650 / BHQ®-2
BODIPY® TMR / **Cyanine-5** (Cy3™)	544	570	BBQ® 650 / BHQ®-2
BODIPY® 564/570	563	569	BBQ® 650 / BHQ®-2
Quasar® 570	548	566	BBQ® 650 / BHQ®-2
Alexa 555	555	565	BBQ® 650 / BHQ®-2
CAL Fluor® Orange 560	538	559	BBQ® 650 / BHQ®-2
HEX	535	556	BBQ® 650 / BHQ®-2
Alexa 532/VIC/BODIPY® 530/550	532	554	BBQ® 650 / BHQ®-2
Rhodamine 6G	528	550	BBQ® 650 / BHQ®-1 (480-580 Abs. Max. 534nm)
Yakima Yellow™	531	549	BHQ®-1
JOE	520	548	BHQ®-1
CAL Fluor® Gold 540	522	544	BHQ®-1
Alexa 430	433	539	BHQ®-1
TET	521	536	BHQ®-1
Rhodamine Green-X	503	528	BHQ®-1
Oregon Green® 514	506	526	BHQ®-1
6-FAM	494	525	BHQ®-1
Alexa 488	495	519	BHQ®-1
Fluorescein/DANSYL	335	518	BHQ®-1
BODIPY® FL	502	513	BHQ®-1
Cy2™	489	506	BHQ®-1
BODIPY® 493/503	493	503	BHQ®-1
Coumarin	432	472	Dabcyl/ Dabsyl/ DDQ-1 (400-550 Abs. Max. 479/ 410nm)
Edans	336	468	Dabcyl/ Dabsyl/ DDQ-1
Acridine	362	462	Dabcyl/ Dabsyl/ DDQ-1
Marina Blue	362	459	Dabcyl/ Dabsyl/ DDQ-1
Pacific Blue	416	451	Dabcyl/ Dabsyl/ DDQ-1
Alexa 350	346	442	Dabcyl/ Dabsyl/ DDQ-1

Data and Protocols

Physical Data

			Dilution (0.1M)/ml			Dilution (0.067M)/ml		
Item No.	Mol. Wt.	Unit Wt.	250mg	500mg	1g	250mg	500mg	1g
1378	713.74	559.67	-	-	-	-	-	-
2426	-	498.49	-	-	-	-	-	-
2427	-	638.57	-	-	-	-	-	-
2550	1079.18	638.57	2.32	4.63	9.27	3.46	6.92	13.83

Protocols

BlackBerry® Quenchers

1378	BBQ-650® *N*-hydroxy-succinimide ester
2427	3'-BBQ-650® CPG II
2550	BBQ-650®-(DMT)-CE-Phosphoramidite

Dissolution

Dilution volumes (in ml) are for 0.1M solutions for **2550**. Adjust accordingly for other concentrations. For µmol pack sizes, products should be diluted as 100µmol/ml to achieve 0.1M, regardless of molecular weight.

2550 is only sparingly soluble in acetonitrile therefore dissolve in anhydrous DCM, AF (7 parts) and once completely dissolved (~10-15min) add anhydrous acetonitrile (3 parts). Do not premix the diluents.

Coupling

2550 – Use a 15min coupling time. Fast deprotection amidites are recommended.

After synthesis, flush the lines with DCM followed by MeCN to remove the dye immediately after use. This will prevent any precipitation or cross contamination.

2427 is used per standard solid supports following the synthesiser instructions. Non-nucleosidic CPG supports do not detritylate as rapidly as nucleosidic ones, therefore an additional detritylation step should be employed. It is therefore necessary to use a cycle that does not contain an initial capping step. Fast deprotection amidites are recommended.

Cleavage & Deprotection

2550 -

1. If the amidite is added to the 5'-end or the 3'-end on universal support. After synthesis, treat the support bound oligonucleotide with 20% DEA in acetonitrile (**4028**) to remove the cyanoethyl protection from the phosphate linkages. This will prevent any loss of the quencher during deprotection.
2. Cleavage and deprotection are achieved in the same step by treating the support bound oligonucleotide with AMA, 10min, 65ºC.

2427 -

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Post-Synthetic Labelling with 1378

Solubility: DMF to 5-10mg/ml or 7-14µmol/ml

Post synthetic labelling protocol:[1]

1. Dissolve the amino functionalised biomolecule in 50mM carbonate buffer (pH 9.3).
2. Dissolve the NHS ester in DMF.
3. Add the solution from step 2 (1 part) to the solution from step 1 (2 parts).
4. Incubate overnight at 30ºC.
5. Quench the reaction with Tris-HCl buffer pH 7.2
6. The conjugated product is now ready for purification.

Spectral Data

Ɛ (M-1cm-1)	40667	15077
λ	598nm	260nm
Solvent	Methanol	

Storage & Stability

Products are stored in light-protected containers in the freezer at –10 to –30°C. The phosphoramidite is susceptible to oxidation when left exposed to air and/or moisture, but is stable in solution under argon for 2 days.

Dabsyl Quencher

2426	3'-Dabsyl-CPG

Coupling

2426 is used as per standard nucleoside supports. However, since non-nucleosidic modifications detritylate at a slower rate it is recommended to carry out an initial additional detritylation. In this case it is important not to carry out any additional capping.

Cleavage & Deprotection

As this has no nucleobase protection, complete the cleavage and deprotection with conditions suitable for the other nucleobases in the oligo. Standard oligonucleotide deprotection conditions can be applied when deprotecting an oligo containing this modification; typically AMA, 55ºC, 35min.

Storage & Stability

Store dry in a freezer at –10 to –30°C.

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1 **A dual-step fluorescence resonance energy transfer-based quenching assay for screening of caspase-3 inhibitors**, A. Valanne, P. Malmi, H. Appelblom, P. Niemelä and T. Soukka, *Anal. Biochem.*, **375**, 71-81, 2008.

Ordering Information

Product	Pack Size	Cat. No.
BlackBerry® Quenchers		
BBQ-650® *N*-hydroxy-succinimide ester	5mg	1378-B005
	25mg	1378-B025
	100mg	1378-B100
3'-BBQ-650® CPG II	100mg	2427-B100
	1g	2427-C001
ALL-FIT Columns*	4 x 0.2µmol	2427-P001
	10 x 0.2µmol	2427-P002
	4 x 1µmol	2427-P010
	10 x 1µmol	2427-P008
BBQ-650®-(DMT)-CE-Phosphoramidite	50µmol	2550-F050
	100µmol	2550-F100
	250mg	2550-B250

Product	Pack Size	Cat. No.
Dabsyl Quencher		
3'-Dabsyl-CPG	100mg	2426-B100
	1g	2426-C001
ALL-FIT Columns*	4 x 0.2µmol	2426-P001
	10 x 0.2µmol	2426-P002
	4 x 1µmol	2426-P010
	10 x 1µmol	2426-P008

* Please enquire about alternative column types and scales.

BlackBerry® Quencher technology: Covered under US Patent #7,879,986, Berry & Associates, Inc. BlackBerry Quencher,' 'BBQ 650 CEP,' 'BBQ 650 CPG' and 'BBQ 650 NHS Ester' are trademarks of Berry & Associates, Inc., MI. The BBQ dye technology is sold under agreement with Berry & Associates, Inc. Products are sold exclusively for R&D use by the end user. They may not be used for clinical or diagnostic purposes.

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Exceptional as standard