GLEN RESEARCH

22825 DAVIS DRIVE
STERLING, VIRGINIA
20164

PHONE

703-437-6191

800-327-GLEN

FAX

703-435-9774

INTERNET

WWW.GLENRES.COM

GUIDANCE ON THE USE OF INTERNALLY QUENCHED NUCLEOTIDE (IQN)

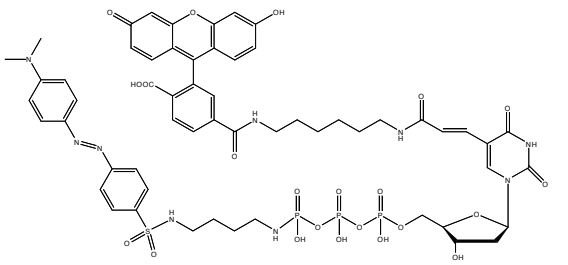
Lawler Scientific LLC., in partnership with Glen Research Corporation, has developed the Internally Quenched Nucleotide – Fluorescein-dUTP-Dabsyl. Several applications of the Internally Quenched Nucleotides (IQN) are in development. Below we present the current conditions for the use of the Internally Quenched Nucleotide[1] (IQN) by various enzymes.

Snake venom phosphodiesterase rapidly hydrolyzes the IQN and serves as a robust positive control for most applications. We have also experimented with a number of polymerases and have found reverse transcriptases such as AMV-RT to be the most permissive of IQN incorporation and extension. Thermophilic DNA polymerases incorporate and extend the IQNs better than mesophilic polymerases. Among the thermophilic polymerases, Deep Vent (exo-) and Vent (exo -) polymerases from New England Biolabs have produced the most consistent results. Various brands of Taq DNA polymerases have been used with IQNs and we have not been able to establish suitable conditions for use with the IQN. Taq polymerases incorporate IQNs less efficiently and are more prone to hydrolyze the triphosphate of the IQNs to yield false positives. (1,2)

Generally, the conditions recommended by the manufacturers of the polymerase were followed when using the IQN although the addition of organic modifiers such as Tween 20 (0.01% final concentration) or DMSO (0-1%) have been used to improve IQN incorporation.

Note that the use of excessive amounts of organic modifiers may interfere with the quenching by interfering with the association of the fluorophore with the quencher. The pH of TRIS buffer is temperature sensitive and drops as the temperature rises. In early experiments, pH was varied in an effort to reduce background signals during PCR. We have found that changing pH has a minimal effect on IQN hydrolysis at elevated temperatures.

Figure 1: Fluorescein-dUTP-Dabsyl



References:

1. Hanaki, K., Odawara, T., Muramatsu, T., Kuchino, Y., Masuda, M., Yamamoto, K., Nozaki, C., Mizuno, K. and Yoshikura, H. (1997) Primer/template-independent synthesis of poly d(A-T) by Taq polymerase. *Biochem Biophys Res Commun*, **238**, 113-118.
2. Hanaki, K., Odawara, T., Nakajima, N., Shimizu, Y.K., Nozaki, C., Mizuno, K., Muramatsu, T., Kuchino, Y. and Yoshikura, H. (1998) Two different reactions involved in the primer/template-independent polymerization of dATP and dTTP by Taq DNA polymerase. *Biochem Biophys Res Commun*, **244**, 210-219.

[1] IQN technology is covered by Patent # 7,118,871 owned by Lawler Scientific.

ORDERING INFORMATION

Catalog number: 88-1056-01, Fluorescein-dUTP-dabsyl (1mM, 25µL 10mM TRIS, 1mM EDTA) see:
http://www.glenres.com/index.html/Catalog/triphosphates.html

For additional information see:
http://www.glenres.com/index.html/GlenReports/GR17-16.html