PCRBIOSYSTEMS
simplifying research

20x dUTP UDGase Additive

Product description:

20x dUTP UDGase is compatible with PCR Biosystems range of real-time PCR mixes. The reagent contains dUTP, stabilizers and uracil DNA glycosylase (UDGase). The dUTP concentration ensures sufficient incorporation for complete removal of contaminating PCR products.

Carry-over contamination can be prevented by incorporating dUTP in all PCR products and treating all subsequent fully preassembled starting reactions with UDGase, followed by thermal inactivation of UDGase. UDGase cleaves the uracil base from the phosphodiester backbone of uracil-containing DNA, but has no effect on natural (i.e., thymine-containing) DNA. The resulting apyrimidinic sites block replication by DNA polymerases, and are very labile to acid/base hydrolysis. Because UDG does not react with dUTP, and is also inactivated by heat denaturation prior to the actual PCR, carry-over contamination of PCRs can be controlled effectively if the contaminants contain uracils in place of or in addition to thymines.

Pack Size	Format	
500 x 20 µl rxns	20x ReadyMix	1 x 500 µl
2000 x 20 µl rxns	20x ReadyMix	4 x 500 µl

Shipping and Storage

On arrival the kit should be stored at -20˚C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4˚C for 1 month. The kit can go through 30 freeze/thaw cycles with no loss of activity.

Limitations of product use

The product may be used only for in vitro research purposes.

Technical support

For technical support and troubleshooting please email technical@pcrbio.com the following information:

Amplicon size
Reaction setup
Cycling conditions
Screen grabs of amplification traces and melting profile

Important considerations

Primer design: For efficient amplification under fast cycling conditions we recommend amplicon lengths between 80bp and 200bp. With all manufacturers master mixes the shorter the amplicon length the faster the reaction can be cycled. Amplicon lengths should not exceed 400bp. Primers should have a predicted melting temperature of around 60˚C, using default Primer 3 settings (http://frodo.wi.mit.edu/primer3/).

Reaction setup

1. Before starting, briefly vortex 2x qPCRBIO SyGreen or qPCRBIO Probe Mix.
2. Prepare a master mix based on following table:

Reagent	20µl reaction	Final concentration	Notes
2x qPCRBIO SyGreen/Probe Mix	10µl	1x	
Forward primer (10µM)	0.8µl	400nM	See above for optimal primer design
Reverse primer (10µM)	0.8µl	400nM	
Probe (10µM)	0.4µl	200nM	For probe reactions only
20x UTP UDGase	1.0µl	1x	
Template DNA	<100ng cDNA, <1µg genomic	variable	
PCR grade dH_2O	Up to 20µl final volume		

3. Program the instrument using following conditions, acquiring data on the SYBR® Green or relevant probe channel:

Cycles	Temperature	Time	Notes
1	37˚C	10min	Incubation for UDGase removal of dUTP containing DNA
1	95˚C	5min	Polymerase activation and denaturation of UDGase
40	95˚C 60˚C to 65˚C	5 seconds 20-30 seconds	Denaturation Anneal/Extension, do not exceed 30 seconds, do not use temperatures below 60˚C
Melt analysis	Refer to instrument instructions		Optional melt profile analysis, available for hybridisation probes only