Product Information Sheet

Delta 2X qPCR Master Mix

Storage and Handling:

Store at -20^{0}C upon arrival. The kit is stable for 1 year from the date of receipt when stored at dark in a constant temperature (-20^{0}C) freezer. Before use, thaw the master mix to room temperature and vortex gently to mix. It can be refrozen in -20^{0}C freezer for storage.

Product Description:

Delta 2X qPCR Master Mix is a ready to use hot-start qPCR master mix with EvaGreen® and DeltaTaq, a hot start Taq polymerase. A 25µM ROX reference dye is supplied separately for use with some real time instruments see Table 1. The master mix is optimized to work with both fast cycling conditions and regular three step cycling conditions.

DeltaTaq, a product of Precision Biosystems, is an exceptionally chemically modified hot-start Taq DNA polymerase that can be activated in as less as 2 minutes making it highly suitable for fast qPCR. DeltaTaq is a better hot-start enzyme compared to other commercially available chemically modified hot-start Taq that require 10mins of activation time. Also, DeltaTaq is better compared to antibody-based hot start Taq that have a high risk of contamination from the organism they were produced in.

EvaGreen® dye is a dsDNA-binding dye with properties ideal for qPCR. High concentrations of EvaGreen® dye can be used in qPCR without inhibiting PCR reactions because of the novel "release-on-demand" mechanism by which the dye binds to dsDNA.

EvaGreen® dye is uniquely safe in comparison to other DNA-binding dyes. Generally, DNA binding dyes are mutagenic and hence are dangerous. It has been shown that SYBR® Green I is even more toxic than Ethidium Bromide (Ohta et al. 2001). EvaGreen® , however is cell membrane impermeable making it non-cytotoxic, non-mutagenic, safe to aquatic life and hence can be directly disposed down the drain. These properties make EvaGreen® a highly preferable dye in qPCR.

The absorption and emission spectra of EvaGreen® dye are similar to SYBR® Green I and FAM: $\lambda_{abs}/\lambda_{em}$ = 500/530 nm when bound to DNA and λ_{abs} = 471 nm when free in the solution. This makes EvaGreen® compatible with all real time instruments that are compatible with SYBR® Green I, without the need to use new filters.

Protocol:

Reaction set-up:

Component	Volume	Final Concentration
Delta 2X qPCR MM	10 µl	1X
Reference Dye (ROX)	X µl	Note 1
Primer	X µl	0.1-1.0µM, Note 2
Template	X µl, Note 4	<250ng
Nuclease Free Water	Add to 20µl	

Notes:

1. *Reference Dye*: ROX reference dye is used with some real time instruments to normalize well-to-well variation in fluorescence. Refer to Table 1 for more details.

2. *Primers*: 0.1-1.0µM final concentrations of forward and reverse primers can be used based on the primer composition. Run a primer efficiency test with different primer concentrations and select the one that shows efficiency closest to 100%.

3. *Amplicon Length*: The optimal amplicon length for qPCR with *delta* 2X qPCR Master Mix is 50-200bp for an efficient reaction. Thermal cycling conditions can be changed based on the amplicon length (if longer amplicons are required).

4. *Template*: The volume of the template should not exceed 10% of the total reaction volume. Both gDNA and cDNA templates can be used with *delta* 2X qPCR Master Mix. Recommended amounts of gDNA and cDNA are between 50pg-50ng and 50fg-50pg respectively based on the amount of RNA used for RT reaction.

Thermal Cycling Conditions:

Two Step fast cycling:

Cycling Step	Temperature	Holding Time	Cycles
Enzyme activation	95^{0}C	2 min	1
Denaturation	95^{0}C	5sec	40
Annealing & Extension	60^{0}C	30sec	

Three Step cycling:

Cycling Step	Temperature	Holding Time	Cycles
Enzyme activation	95^{0}C	2 min	1
Denaturation	95^{0}C	5sec	40
Annealing	55-60^{0}C	5sec	
Extension	72^{0}C	25sec	

Dissociation program:

Temperature	Holding Time	Cycles
95^0C	1min	1
55^0C	30sec	
95^0C	30sec	

Table 1: ROX concentration recommendation for different instruments

Type	Instrument		ROX final concentration
	Company	Instrument Name	
No ROX	Roche	LightCycler 480, LightCycler 2.0	
	BioRad	iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384, Chromo4, MJ Opticon, Option2, MiniOpticon	
	Qiagen	Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000	
	Illumina	Eco RealTime PCR System	
	Eppendorf	Mastercycler realplex	
	Cepheid	SmartCyler	
Low ROX	ABI	7500, 7500 Fast	30nM
	Stratagene	MX4000P, MX3000P, MX3005P	
High ROX	ABI	5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus, Viia7	300nM

References:

1. Mao, et al. Characterization of EvaGreen Dye and the implication of its physicochemical properties for qPCR applications. BMC Biotechnology 7, 76 (2007).

2. Ohta, et al. Ethidium bromide and SYBR Green I enhance the genotoxicity of UV-irradiation and chemical mutagens in E. coli. Mutation Res. 492, 91-97 (2001).

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