

# DyNAmo™ Flash Probe qPCR Kit

## DyNAmo™ ColorFlash Probe qPCR Kit

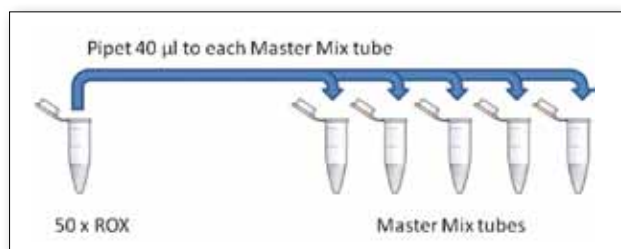
### Instructions for Applied Biosystems real-time PCR instruments

For complete instructions and additional information please refer to the instruction manual.

#### Addition of ROX™ passive reference dye

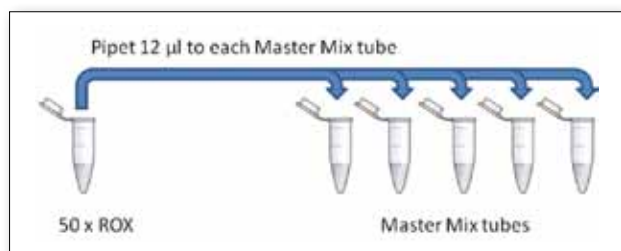
##### ABI 7000, 7300, 7700 and 7900: 1x ROX final concentration

1. Thaw and mix 50x ROX and 2x Master Mix tubes carefully.
2. Add 40 µl of 50x ROX to each 1 ml 2x Master Mix tube.
3. Mix again carefully.
4. Store at -20°C.



##### ABI 7500, StepOne™: 0,3x ROX final concentration

1. Thaw and mix 50x ROX and 2x Master Mix tubes carefully.
2. Add 12 µl of 50x ROX to each 1 ml 2x Master Mix tube.
3. Mix again carefully.
4. Store at -20°C.



#### Reaction setup for all ABI models:

Step 3 (adding sample buffer) is optional. You can perform it if you are using DyNAmo ColorFlash SYBR Green qPCR Kit (F-416) and wish to track pipetting when adding samples to the reactions.

1. Program the cyclers as outlined in Table 2.
2. Thaw template DNA, primers and 2x master mix (where ROX passive reference dye has been added). Mix the individual solutions to assure homogeneity. This is especially important for the master mix.
3. If using a DyNAmo ColorFlash qPCR Kit, add the yellow sample buffer to the samples. Add the buffer to a

concentration that will dilute to 1x in the final reaction volume. For example, if 5 µl of sample is to be used in a 20 µl reaction volume, 4x buffer concentration in the sample results in 1x buffer concentration in the final reaction.

4. Prepare a PCR premix by mixing 2x master mix, primers, and H<sub>2</sub>O. Mix the PCR premix thoroughly to assure homogeneity. Dispense appropriate volumes into strip tubes or plate wells. Use the reverse pipetting technique to avoid bubbles.
5. Add template DNA (< 200 ng per 20 µl reaction) to the strip tubes or plate wells containing the PCR premix. For two-step qRT-PCR, the volume of the cDNA added (from the RT reaction) should not exceed 10 % of the final PCR volume.
6. Seal the strips or plate with an appropriate sealer, place them in the thermal cycler and start the cycling program.

**Table 1: Reaction setup for hydrolysis probes (TaqMan®, Double Dye, etc.).**

Components (In order of addition)	50 µl reaction	20 µl reaction	Final conc.	Comments
2x master mix	25 µl	10 µl	1x	Mix thoroughly.
Primer mix (in H <sub>2</sub> O)	X µl	X µl	500 nM fwd 500 nM rev	Titrate from 50 to 1000 nM, if necessary.
Probe	X µl	X µl	250 nM (TaqMan® probe)	Titrate from 50 to 500 nM, if necessary.
Template DNA (including yellow sample buffer)	X µl	X µl		In general, max 200 ng/20 µl reaction. Using the yellow sample buffer is optional.
H <sub>2</sub> O	add to 50 µl	add to 20 µl		

**Table 2: qPCR protocol**

Step	Temp.	Time	Cycles
Initial denaturation	95°C	7 min	1
Denaturation	95°C	5 s	40 cycles
Annealing/extension	60°C	30 s *	

\* A shorter annealing/extension (down to 15 s) step can be used with the following instruments: ABI 7000, 7700 and 7900. For additional information please refer to the DyNAmo™ Flash Probe qPCR Kit (F-455 and F-456) instruction manual.