

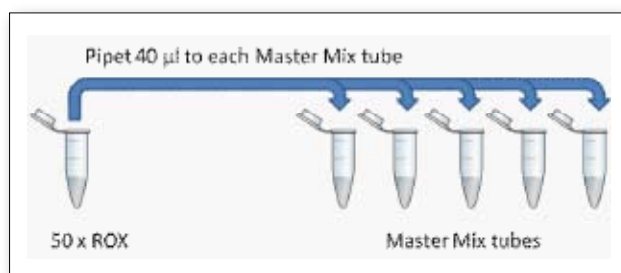
# DyNAmo™ HS SYBR® Green qPCR Kit

## Instructions for Applied Biosystems real-time PCR instruments

### Addition of ROX™ passive reference dye

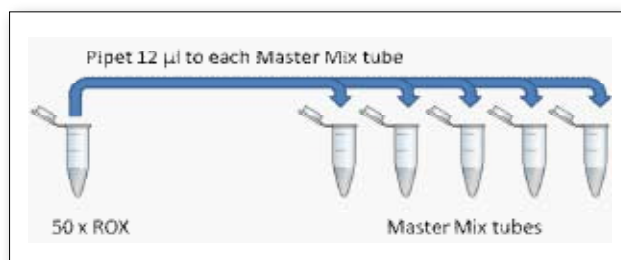
#### ABI 7000, 7300, 7700 and 7900: 1x ROX final concentration

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.



### DyNAmo HS SYBR® Green protocol for all ABI models:

1. Program the cyclor 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. 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 reverse pipeting technique to avoid bubbles.
4. Add template DNA (< 200 ng/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.
5. Seal the strips or plate with appropriate sealer, place them in the thermal cyclor and start the cycling program.

**Table 1: Reaction setup**

Components (In order of addition)	Volume / 50 µl reaction	Volume / 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	0.5 µM fwd 0.5 µM rev	Titrate from 0.3 to 1 µM, if necessary.
Template DNA (in H <sub>2</sub> O)	X µl	X µl		Do not exceed 10 ng/µl in the final reaction.
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	15 min	1
Denaturation	95°C	10 s	40 cycles
Annealing/extension	60°C	60 s	
Dissociation curve as instructed by the instrument manufacturer			

NOTE: Make sure to design primers suitable for 2-step protocol. For a 3-step protocol and additional information please refer to the DyNAmo™ HS SYBR® Green qPCR Kit (F-410) instruction manual.