

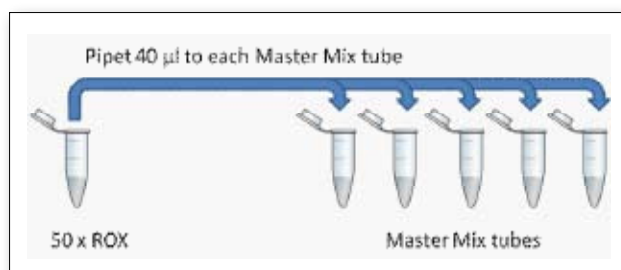
DyNAmo™ Flash Probe qPCR Kit

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™ Flash Probe qPCR protocol for all ABI models:

1. Program the cyclers as outlined in Table 2.
2. Thaw template DNA, primers, probe(s) 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, probe(s) and H₂O. Mix the PCR premix thoroughly to assure homogeneity. Dispense appropriate volumes into strip tubes or plate wells. Use reverse pipetting 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 cycler and start the cycling program.

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

| 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 ₂ 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 (in H ₂ O) | X µl | X µl | | In general, max 200 ng/20 µl reaction. |
| H ₂ 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) instruction manual.