

Protocol For Making 100 μ M Primer Stocks

When the primers arrive, punch holes in the Specification Sheets and put them in the appropriate notebook. Primer tubes from IDT* will indicate the OD₂₆₀, nmol, and mg along the bottom of the label. Spin down the tubes before opening so that no primer falls out. To make a 100 μ M solution simply add 10 times the amount of water as nmol of your primer.

The label will look like this:

$$5.6 \text{ OD}_{260} = 28.18 \text{ nmol} = 0.18 \text{ mg}$$

For example, you will add ($28.18 \times 10 =$) 281.8 μ l of water for this primer. This gives a 100 μ M stock. Vortex each tube after adding water and let stand on the bench for 15 minutes. Vortex again before using to make working solution. To make a working solution, add 12.5 of the forward and 12.5 of the reverse to 975 μ l of water for 1 ml of working solution primer.

In order to preserve our primers for longer, aliquot it out and dry it down in the speed vac (with the **heat off**). To do this, put 12.5 μ l of the forward and 12.5 μ l of the reverse primer in a 1.5 ml tube. If possible, aliquot out all of the primer in this manner. Place tubes in the speed vac and turn on the vacuum pump and the rotor. It may take several hours for them to dry down. Once dry, store them at -20 C. When ready to use, add 1 ml of water to a tube, vortex and let stand 15 min at room temp before using. This will give a 25 μ M working solution.

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* The blue (HEX) and green (6-FAM) dye colors can be ordered from our usual primer supplier, IDT. For the yellow dye color (NED) we have only been able to order from Applied Biosystems, which does not label their primers in this way.
