

dNTP Aliquots

Reagents

| Reagent | Vendor | Catalogue # |
|-------------------------------------|------------|-------------|
| dGTP, dATP, dCTP, dTTP (100mM each) | Bio-Budget | 80-85011000 |
| ddH ₂ O | | |

Since many different people in the lab will be using these aliquots for their PCR reactions, it is important to minimize any possible DNA contamination during the procedure. Wear a lab coat and gloves, as well as work within in the PCR workstation.

dNTPs are sensitive to hydrolysis on the phosphate groups and thus must be kept on ice whenever possible. Do not remove from ice until ready.

1. Thaw each nucleotide on ice.
2. While they are thawing, turn on the UV lamp in the PCR workstation for at least 20 minutes
3. Once you can no longer see ice in the tube, vortex each tube and, if necessary, centrifuge them briefly to bring the liquid to the bottom
4. Bring the dNTPs to the PCR workstation, but keep them in the ice bucket next to it until you are ready to work with them.
5. Label 40 sterile, DNase free 0.67mL Eppy tubes with "10mM dNTP"
6. In a sterile, DNase free 1.7mL Eppy tube, add the following components:

| Reagent | Stock Conc. | Vol (µl) | Final Conc. |
|--------------------|-------------|----------|-------------|
| dATP | 100mM | 25 | 2.5mM |
| dCTP | 100mM | 25 | 2.5mM |
| cGTP | 100mM | 25 | 2.5mM |
| dTTP | 100mM | 25 | 2.5mM |
| ddH ₂ O | - | 900 | |

7. Vortex the solution
8. Aliquot 25µl of the 10mM (2.5mM each dNTP) mix into each of the 40 tubes
 - a. Transfer each tube to ice **immediately**
9. Transfer aliquots to a box and label the box "10mM dNTP" as well your initials and date
10. Store at -20°C in the Molecular Lab.