Nucleotide preparations (100mM)

By following the procedure below you should end up with a large stock of free nucleotides that can be used for sequencing, PCR, random hexamer/oligo nucleotide labeling, etc. And it is real cheap! For additional info see also Sambrook Appendix B10.

Resuspend the entire vial into the indicated volume of sterile ddH_2O (or 1X $T_{10}E_{0.1}$).

Nucleotide	Quantity in Sigma vial	Volume ddH_2O
dATP (Sigma D 6500)	25mg	400µl
dGTP (Sigma D 4010)	10mg	160µl
dCTP (Sigma D 4635)	25mg	400µl
dTTP (Sigma T 0251)	25mg	400µl

Bring the pH of each nucleotide to ~7. To do this Sambrook recommends adding 1M Tris. I have found that adding 10µl of 1M Tris is insufficient to raise the pH to 7. Therefore I add 10µl of 1M Tris and then 1µl aliquots of 10N NaOH until the pH reaches about 7. It takes 2 to 4µl of 10N NaOH to do this. To determine the pH, simply spot 10µl aliquots onto pH paper.

Determine the exact concentration of each nucleotide by first diluting 1.0µl of the dNTP into 2000µl TE (0.5µl into 1000µl) and measuring the OD at the appropriate wavelength (λ). You then need to divide λ by the extinction coefficient (ε). For a cuvette with a 1cm path length (which we assume), absorbance (A) at the $\lambda = \varepsilon M$ where M is the molarity.

Nucleotide	λ	Extinction coefficient (ε) ($M^{-1} \cdot cm^{-1}$)
dATP	259	1.54×10^4
dGTP	253	1.37×10^4
dCTP	271	9.10×10^3
dTTP	260	7.40×10^3

For example: Lets say that you diluted 0.5µl of dATP into 1000µl. The OD_{260} is then determined to be 0.6922.

$M = \lambda/\varepsilon$
$M = (0.6922 \times 2000)/(1.54 \times 10^4)$
$M = 0.090 =$ **90mM**

Store at –80°C. Repeated freezing and thawing is not good for nucleotides so Sambrook recommends aliquoting the dNTPs into small volumes.

Materials Needed:

Nucleotides
1M Tris
10N NaOH
pH paper
Spectrophotometer