Primary application	Compound	Specific activity (Ci/mmol)	Radiochemical concentration (mCi/mL)	Molar concentration (µM)	Catalog number for color-coded EasyTides version; Shipped ambient, Store at 2-8°C	Cat. No. for frozen version; store at -20°C
DNA labeling (Random primer or nick translation) or DNA sequencing	dATP,[alpha- <sup>32</sup> P]	800	10	12.5	BLU512A/NEG512A	
(Klenow, T7 DNA polymerase, terminal transferase)		3000	10	3.3	BLU512H/NEG512H	BLU012H/NEG012H
		6000	20	3.3	BLU512Z/NEG512Z	BLU012Z/NEG012Z
	dCTP,[alpha- <sup>32</sup> P]	800	10	12.5	BLU513A/NEG513A	BLU013A
		3000	10	3.3	BLU513H/NEG513H	BLU013H/NEG013H
		6000	20	3.3	BLU513Z/NEG513Z	BLU013Z/NEG013Z
	dGTP,[alpha- <sup>32</sup> P]	3000	10	3.3	BLU514H/NEG514H	
		6000	20	3.3	BLU514Z/NEG514Z	BLU014Z/NEG014Z
	dTTP,[alpha- <sup>32</sup> P]	800	10	12.5	BLU505A/NEG505A	BLU005A/NEG005A
		3000	10	3.3	BLU505H/NEG505H	BLU005H/NEG005H
	dATP,[alpha- <sup>33</sup> P]	3000	10	3.3	NEG612H	NEG312H
	dCTP,[alpha- <sup>33</sup> P]	3000	10	3.3	NEG613H	NEG313H
	dTTP,[alpha- <sup>33</sup> P]	3000	10	3.3	NEG605H	
3' end labeling of DNA using terminal transferase	3' dATP,[alpha- <sup>32</sup> P]	5000	10	2		BLU026/NEG026

See next page for information on how to select a radionucleotide from this table.

Guidelines for choosing a deoxynucleotide from the above table:

- Application
  - dNTPs (deoxynucleoside triphosphates) that are labeled on the alpha phosphate group are typically used in reactions involving enzymes that will
    incorporate the deoxynucleoside monophosphate (base, sugar, and alpha phosphate) into a chain of DNA.
  - 3' end labeling of DNA typically utilizes chain terminator, such as dATP that is deoxy at the 3' position, to prevent further elongation once the radionucleotide has been incorporated (controls the degree of labeling). However, "tailing reactions" can also be performed to insert multiple nucleotides at the 3' end of a piece of DNA using a radionucleotide that is not a chain terminator.
- Compound
  - Radionucleotides are labeled with either <sup>32</sup>P, <sup>33</sup>P, or <sup>35</sup>S radioisotope. <sup>32</sup>P is a high energy beta emitter, and will produce the highest signal. <sup>33</sup>P and <sup>35</sup>S are considered low energy beta emitters, and are sometimes used instead of <sup>32</sup>P to improve resolution, especially in sequencing reaction and *in situ*.
  - Radiolabled dATP, dCTP, dGTP, and dTTP are offered. If your reaction is template-dependent, you may need to refer to the sequence of your template to choose the best radionucleotide for your assay. Some enzymatic assays are template-independent (such as end labeling with terminal transferase), and it will not matter whether the radionucleotide is a dATP, a dCTP, etc.
  - 3' dATP is a chain terminator, because it is deoxy- (missing an oxygen) at the 3' position. It is often used in lieu of ddATP.
- Specific activity
  - Specific activity indicates how much radioactivity there is per molecule. The units for specific activity in the table above are Curies per millimole of nucleotide. The theoretical maximum specific activity for <sup>32</sup>P is ~9120 Ci/mmol. The theoretical maximum specific activity for <sup>33</sup>P is ~5000 Ci/mmol. The theoretical maximum specific activity for <sup>35</sup>S is ~1488 Ci/mmol. Because the nucleotides in this table have only one possible labeling position, the closer the specific activity is to the theoretical maximum specific activity for the radioisotope, the greater the proportion of nucleotide molecules that are labeled with the radioisotope in the stock vial. Remember to <u>factor in decay</u>.
  - If you are trying to generate "hot" probes, you will want to choose a radionucleotide with a high specific activity.
  - Specific activity can always be decreased by adding more of the same "cold" (unlabeled) nucleotide. This will increase the molar concentration of the nucleotide.
- Radiochemical concentration
  - Radiochemical concentration indicates the amount of radioactivity per volume. If your protocol tells you to add a certain amount of Curies to a reaction, you will need to use the radioactive concentration to determine how much to pipette. Remember to <u>factor in decay</u>.
- Molar concentration refers to the molar concentration of both the labeled and unlabeled nucleotide (combined) in the stock vial.
- Catalog numbers
  - You have up to four choices for each radionucleotide. These differ by:
    - Container: <u>BLU</u> products are packaged in a lead-free container ("pig"). <u>NEG</u> products are packaged in a lead-lined container (plastic pig container a layer of lead). Lead provides more shielding from beta energy, but beta particles can interact with lead to generate Bremsstrahlung X-rays (Bremsstrahlung effect). You should talk to your radiation safety officer regarding container selection.
    - Formulation: <u>EasyTides</u> products are provided in a proprietary buffer that contains a dye to aid in pipetting, and can be stored at 4°C (avoiding freeze-thaw cycles). The EasyTides proprietary buffer does contain a somewhat higher concentration of salt, so you may want to choose a non-EasyTides (frozen) formulation if your enzyme is sensitive to salt (thermophilic polymerases for PCR such as Taq). <u>Frozen</u> products do <u>not</u> contain a dye in the buffer, and should be aliquotted (to avoid freeze/thaw cycles) and stored at -20°C.