Ribonucleotides (RNA nucleotides)

Primary application	Compound	Specific activity (Ci/mmol)	Radiochemical concentration (mCi/mL)	Molar concentration (µM)	Cat. No. for EasyTides version; Shipped ambient, Store at 2-8°C	Cat. No. for frozen version; store at -20°C
RNA labeling (SP6, T3, T7 RNA polymerase)	ATP,[alpha-^{32}P]	800	10	12.5		BLU003X/NEG003X
		3000	10	3.3	BLU503H/NEG503H	BLU003H/NEG003H
	CTP,[alpha-^{32}P]	800	10	12.5	BLU508X/NEG508X	BLU008X/NEG008X
		3000	10	3.3	BLU508H/NEG508H	BLU008H/NEG008H
	GTP,[alpha-^{32}P]	800	10	12.5		BLU006X/NEG006X
		3000	10	3.3	BLU506H/NEG506H	BLU006H/NEG006H
	UTP,[alpha-^{32}P]	800	10	12.5	BLU507X/NEG507X	BLU007X/NEG007X
		800	20	25	BLU507T/NEG507T	
		800	40	50	BLU507C/NEG507C	BLU007C/NEG007C
		3000	10	3.3	BLU507H/NEG507H	BLU007H/NEG007H
		6000	40	6.7	BLU507Z/NEG507Z	BLU007Z/NEG007Z
	ATP,[alpha-^{33}P]	3000	10	3.3	NEG603H	
	CTP,[alpha-^{33}P]	3000	10	3.3	NEG608H	
	GTP,[alpha-^{33}P]	3000	10	3.3	NEG606H	
	UTP,[alpha-^{33}P]	3000	10	3.3	NEG607H	NEG307H
	ATP,[alpha-^{35}S]	1250	12.5	10		NEG033H
	CTP,[alpha-^{35}S]	1250	12.5	10		NEG064H
		1250	70	56		NEG064C
	UTP,[alpha-^{35}S]	800	40	50		NEG039C
		1250	12.5	10	NEG739H	NEG039H
3' end labeling of RNA using T4 RNA ligase	pCp,[5'-^{32}P]	3000	10	3.3	-	BLU019A/NEG019A
5' end labeling of DNA or RNA (T4 PNK)	ATP,[gamma-^{32}P]	10	2	200		BLU002/NEG002
		3000	5	1.7	BLU502H/NEG502H	BLU002H/NEG002H
		3000	10	3.3	BLU502A/NEG502A	BLU002A/NEG002A
		6000	10	1.7	BLU502Z/NEG502Z	BLU002Z/NEG002Z
		6000	150	25		NEG035C
	ATP,[gamma-^{33}P]	3000	10	3.3	NEG602H	NEG302H
	ATP,[gamma-^{33}P] for high-throughput	3000	10	3.3	NEG602K	

Guidelines for choosing a ribonucleotide from the above table:

- Application
  - rNTPs (ribonucleoside triphosphates) that are labeled on the alpha phosphate group are typically used in reactions involving enzymes that will incorporate the ribonucleoside monophosphate (base, sugar, and alpha phosphate) into a chain of RNA.
  - 3' end labeling of RNA typically utilizes chain terminator (such as 5'- ^{32}P-pCp with T4 RNA ligase) 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 RNA using a radionucleotide that is not a chain terminator.
- Compound
  - Radionucleotides are labeled with either ^{32}P, ^{33}P, or ^{35}S radioisotope. ^{32}P is a high energy beta emitter, and will produce the highest signal. ^{33}P and ^{35}S are considered low energy beta emitters, and are sometimes used instead of ^{32}P to improve resolution.
  - Radiolabled ATP, CTP, GTP, and UTP 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, and it will not matter whether the radionucleotide is an ATP, a CTP, etc.
  - 5'-pCp is a chain terminator because there is a phosphate group at the 3' position of the sugar, rather than a hydroxyl group. The 5' phosphate group of pCp is labeled with ^{32}P.
  - ^{33}P-gamma-ATP for high-throughput screening (NEG602K) contains a stabilizer in the buffer that allows you to keep the radiochemical at room temperature for longer periods of time while you are setting up plates
- 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 ^{32}P is ~9120 Ci/mmol. The theoretical maximum specific activity for ^{33}P is ~5000 Ci/mmol. The theoretical maximum specific activity for ^{35}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 factor in decay.
  - 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 factor in decay.
- Catalog numbers
  - You have up to four choices for each radionucleotide. These differ by:
    - Container: BLU products are packaged in a lead-free container ("pig"). NEG 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: EasyTides 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 very sensitive to salt. Frozen products do not contain a dye in the buffer, and should be aliquotted (to avoid freeze-thaw cycles) and stored at -20°C.