Radionucleotides for kinase, 5’ end labeling, and other assays

Primary application	Compound	Specific activity (Ci/mmol)	Radiochemical concentration (mCi/mL)	Molar concentration (uM)	Catalog number for color-coded EasyTides version; Shipped ambient, Store at 2-8°C	Cat. No. for frozen version; store at -20°C
Protein phosphorylation, 5' end labeling of DNA or RNA (T4 PNK, protein kinase)	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 screening	3000	10	3.3	NEG602K	
	GTP,[gamma-^{32}P]	6000	10	1.7	BLU504Z/NEG504Z	BLU004Z/NEG004Z
Protein thiophosphorylation	ATP,[gammaS^{35}S]	25-100	12.5	125-500		NEG027
		1250	12.5	10		NEG027H
Cycle sequencing	GTP,[gamma-^{33}P]	3000	10	3.3	NEG604H	
Adenyl and guanyl cyclase assays	ATP,[alpha-^{32}P]	800	10	12.5		BLU003X
		300	10	3.3	BLU503H	BLU003H
	GTP,[alpha-^{32}P]	800	10	12.5		BLU006X
ADP ribosylation of proteins	NAD,[^{32}P]	800	5	6.3		BLU023X/NEG023X
GTP binding/G Protein/P2Y receptor studies	GTPgammaS,[^{35}S] (non-hydrolyzable)	1250	12.5	10		NEG030H
		1250	1	0.8		NEG030X

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

Guidelines for choosing a ribonucleotide from the above table:

- Application
  - Protein thiophosphorylation is a protein phosphorylation technique (for protein kinases) that uses a ^{35}S-labeled γATP. The reaction is the same as for protein phosphorylation assays using ATPγ ^{32}P or ^{33}P.
  - Cycle sequencing reactions measure the dissociation of the gamma phosphate group of GTP during hydrolysis of GTP to GDP.
  - Adenyl and guanyl cyclase assays measure the conversion of radioactive ATP or GTP to radioactive cAMP or cGMP, respectively.
  - ADP ribosylation measures ADP-ribosylated proteins by incorporation of radiolabeled NAD
  - GTP binding assays are functional assays for GPCRs, and measure exchange of GDP for radiolabeled, non-hydrolyzable GTP analog upon activation.
- 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 high signal. ^{33}P and ^{35}S are considered low energy beta emitters, and are sometimes used instead of ^{32}P to improve resolution.
  - ^{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.
- 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.
- 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: 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.