Product Data Sheet

Product Name: 4-Thio-UTP
Cat. No.: GB20114

Chemical Properties

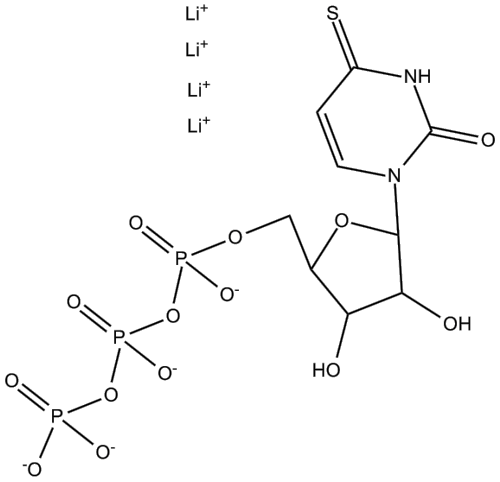
Cas. No.

Formula	$C_9H_{15}N_2O_{14}P_3S$ (free acid)	M.Wt	500.2 (free acid)
Solubility		Storage	Store at -20°C or below

General tips: For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.

Shipping Condition: Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.

Structure



Protocol

Cell experiment [1]:

Cell lines: rat aortic smooth muscle cells

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Preparation Method	Rat aortic VSMCs from both spontaneously hypertensive (SHR) and normotensive (WKY) animals were prepared according to protocol. For the total [^{3}H]-inositol (poly)phosphate measurements, cells were culturedto 80% confluence in 24-well multi-well plates. The medium was then replaced with 500µl of serumfree M199 medium supplemented with 50IU/ml penicillin, 50µg/ml streptomycin, 2mM L-glutamine and1µCi/ml(0.037MBq/ml) D-myo-[2-3H]inositol. After 24h, cells were incubated with 10mM LiCl for 10min – where appropriate suramin was included with the lithium. After 20min stimulation with 4-Thio-UTP (0.1-300µM) in the continuing presence of lithium, the reaction was stoppedwith trichloroacetic acid, the solution was neutralised with freon : octylamine extraction, and addition of $NaHCO_3$ and[^{3}H]inositol phosphates separatedon small Dowex-1 columns.
Reaction Conditions	0.1-300µM; 20min
Applications	4-Thio-UTP evoked a concentration-dependent accumulation of [^{3}H]inositol polyphosphates in cultured rat aortic smooth muscle cells.

Animal experiment [2]:

Animal models	P2Y2$^{-/-}$ and P2Y4$^{-/-}$ mice; DF508 mice

Product Data Sheet

Preparation Method	P2Y2$^{-/-}$ and P2Y4$^{-/-}$ mice and their littermates were maintained on a standard diet in conventional facilities. DF508 mice and their littermates were weaned to a liquid diet and the colony was maintained in a pathogen-free status. Mice were kept in a 12-h light–dark cycle. chloride secretion was assessed as the *Isc* following addition of phloridzin or amiloride to the apical side as well as of indomethacin (100μM) to both bathing media. 4-Thio-UTP was added, at 100μM, to the apical or basolateral solution, unless otherwise stated. Forskolin was always added to the basolateral solution at the concentration of 10μM in ethanol 0.1%; this concentration of ethanol does not affect *Isc*. The increase in *Isc* was calculated as the difference between the basal current and the peak current obtained within 15min of addition of 4-Thio-UTP or forskolin. Statistical analysis was performed using the unpaired t-test and a P-value
Dosage form	100μM; 15min; basolateral perfusion
Applications	4-Thio-UTP stimulated chloride secretion in wild-type mouse jejunum and the response was attenuated in both P2Y4- and P2Y2-deficient mice.

References:

[1] Kumari R, Goh G, Ng LL, Boarder MR. ATP and UTP responses of cultured rat aortic smooth muscle cells revisited: dominance of P2Y2 receptors. *Br J Pharmacol*. 2003;140(7):1169-1176.
[2] Ghanem E, Robaye B, Leal T, et al. The role of epithelial P2Y2 and P2Y4 receptors in the regulation of intestinal chloride secretion. *Br J Pharmacol*. 2005;146(3):364-369.

Background

4-Thio-UTP is a UTP analog and potent $P2Y_2$ and $P2Y_4$ agonist with EC_{50} values of 35 and 350nM for $hP2Y_2$ and $hP2Y_4$, respectively[1]. P2Y2 and P2Y4 are G-protein-coupled purinergic receptors activated extracellularly by UTP that mobilize intracellular Ca^{2+} and thereby regulate epithelial ion/water transport, immune cell recruitment and vascular tone[2]. 4-Thio-UTP is widely used to

investigate purinergic signaling in inflammation, epithelial ion transport, neurotransmission, and immune regulation[3]. 4-Thio-UTP is also used in cross-linking experiments and for labeling transcription complexes to explore the behavior of RNA polymerase during transcription elongation and termination[4].

In vitro, 4-Thio-UTP (0.1-300μM; 20min) evoked a concentration-dependent accumulation of [^{3}H]inositol polyphosphates in cultured rat aortic smooth muscle cells[5].

In vivo, 4-Thio-UTP (100μM; 15min; basolateral perfusion) stimulated chloride secretion in wild-type mouse jejunum and the response was attenuated in both P2Y4-and P2Y2-deficient mice[6]. 4-Thio-UTP (0.44mg/kg; i.v.; 30min before LAD ligation) reduced myocardial infarct size and improved left-ventricular fractional shortening in wild-type mice but lost protection in P2Y2$^{-/-}$mice[7].

References:
[1] Jacobson KA, Costanzi S, Ivanov AA, et al. Structure activity and molecular modeling analyses of ribose- and base-modified uridine 5'-triphosphate analogues at the human P2Y2 and P2Y4 receptors. *Biochem Pharmacol*. 2006;71(4):540-549.
[2] Hede SE, Amstrup J, Klaerke DA, Novak I. P2Y2 and P2Y4 receptors regulate pancreatic Ca(2+)-activated K+ channels differently. *Pflugers Arch*. 2005;450(6):429-436.
[3] Lazarowski ER, Boucher RC. UTP as an extracellular signaling molecule. *News Physiol Sci*. 2001;16:1-5.
[4] Bartholomew B, Dahmus ME, Meares CF. RNA contacts subunits IIo and IIc in HeLa RNA polymerase II transcription complexes. *J Biol Chem*. 1986;261(30):14226-14231.
[5] Kumari R, Goh G, Ng LL, Boarder MR. ATP and UTP responses of cultured rat aortic smooth muscle cells revisited: dominance of P2Y2 receptors. *Br J Pharmacol*. 2003;140(7):1169-1176.
[6] Ghanem E, Robaye B, Leal T, et al. The role of epithelial P2Y2 and P2Y4 receptors in the regulation of intestinal chloride secretion. *Br J Pharmacol*. 2005;146(3):364-369.
[7] Cohen R, Shainberg A, Hochhauser E, et al. UTP reduces infarct size and improves mice heart function after myocardial infarct via P2Y2 receptor. *Biochem Pharmacol*. 2011;82(9):1126-1133.