

Puromycin

Selective antibiotic for the *pac* gene

Catalog # ant-pr-1, ant-pr-5

For research use only

Version # 11H28-MM

PRODUCT INFORMATION

Content:

Puromycin hydrochloride is supplied as 2ml tubes of a 10mg/ml solution in HEPES buffer (100% active product), filtered to sterility for customer convenience, and cell culture tested.

- ant-pr-1: 5 x 2ml at 10 mg/ml (100 mg)
- ant-pr-5: 25 x 2ml at 10 mg/ml (500 mg)

Storage and stability:

Puromycin is shipped at room temperature. Upon receipt it should be stored at -20°C for long term storage and at 4°C for short term storage.

Puromycin is stable for at least three months at 4°C and at least 1 year at -20°C.

Quality control

Purity controlled by HPLC: >95%

Activity controlled by bioassays on bacteria and mammalian cell lines.

SPECIAL HANDLING

Puromycin is a harmful compound. Handle with care.

BACKGROUND

Puromycin is an aminonucleoside antibiotic produced by *Streptomyces alboniger*. It specifically inhibits peptidyl transfer on both prokaryotic and eukaryotic ribosomes. The antibiotic inhibits the growth of Gram positive bacteria and various animal and insect cells. Fungi and Gram negative bacteria are resistant due to the low permeability to the antibiotic. But in some particular conditions puromycin can be used for *E. coli*. For more than 30 years, puromycin has been widely used as a basic tool for studying protein synthesis. Now, puromycin hydrochloride is particularly useful for the selection of cell types harbouring plasmids carrying puromycin resistance genes.

CHEMICAL PROPERTIES

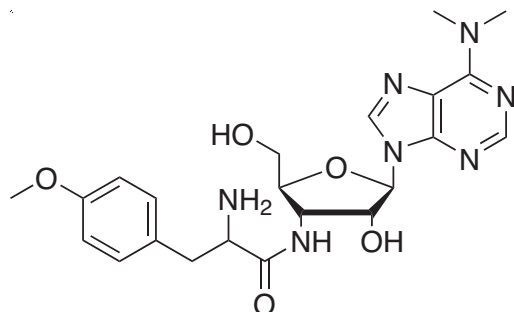
CAS n°: 58-58.2

Formula: C₂₂H₂₉N₇O₅, 2HCl

Molecular weight: 544.3

pKa values: 6.8 and 7.2

Structure:



RESISTANCE TO PUROMYCIN

The *pac* gene encoding a Puromycin N-acetyl-transferase (PAC) has been isolated from a *Streptomyces* producing strain^{1,2}. It is located in a region of the *pur* cluster linked to the other genes determining the puromycin biosynthetic pathway.

The expression of *pac* gene confers puromycin resistance to transfected mammalian cells³ expressing it. In some particular conditions puromycin could also be used for selection of *E. coli* strains transformed with plasmids carrying the *pac* gene.

CONDITIONS OF SELECTION

Puromycin is poorly active on *E. coli* but is particularly useful in experiments conducted with mammalian cells. It can be used as an alternative to Neomycin system for transfection experiments.

Mammalian cells

The working concentrations of puromycin for mammalian cell lines range from 1 to 10 µg/ml. In a starting experiment we recommend to determine optimal concentrations of antibiotic required to kill your host cell line. Puromycin quickly kills eukaryotic cells that do not contain the *pac* gene. Dying cells detach from the plates allowing easy and early identification of transformant clones. Suggested working conditions for selection in some mammalian cells are listed below:

Cell line	Species	Tissue	Culture medium	Puromycin µg/ml
HeLa	Human	Uterus	DMEM	3
293	Human	Kidney	DMEM	3
B16	Mouse	Melanoma	RPMI	1-3
PC1.0	Hamster	Adenocarcinoma	RPMI	10

METHOD (Selection procedure for mammalian cells)

Puromycin is normally used at a concentrations ranging from 1 to 10 µg/ml. After transformation with a plasmid containing the *pac* gene, cells are incubated in their regular growth medium containing Puromycin to select for stable transfectants.

1- 48 hours post-transfection, pass cells (direct or diluted) in fresh medium containing Puromycin at the appropriate concentration.

Note: Antibiotics work best when cells are actively dividing. If the cells become too dense, the antibiotic efficiency will decrease. It is best to split cells such that they are not more than 25% confluent.

2- Remove and replace antibiotic containing medium every 3-4 days.

3- Evaluate cells for the formation of foci after 7 days of selection. Foci may require an additional week or more to develop depending on the host cell line and transfection/selection efficiency.

4- Transfer and pool 5-10 resistant clones to a 35mm cell culture plate and maintain on selection medium for an additional 7 days. This pooled culture will be expanded for subsequent cytotoxicity assays.

References:

- 1- VARA J., et al. (1985). *Biochemistry*. 24: 8074-8081
- 2- LACALLE R.A., et al. (1989). *Gene*. 79: 375-380
- 3- DE LA LUNA S and J.ORTIN. (1992). *Methods In Enzymology*.216:376-385

TECHNICAL SUPPORT

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