GenTarget Inc

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Puromycin Antibiotic Solution

(Research Use Only, Not for Drug Use)

Cat#	Product Name	Amounts
P-anti	Puromycin solution in PBS, cell culture ready	1.0 ml (5.0 mg/ml)

Storage: Upon received, it should be stored at 4°C-20°C. Stable for 6 months.

Product Description:

Puromycin is an antibiotic protein synthesis inhibitor which causes premature chain termination during translation. It derived from the *Streptomyces alboniger* bacterium.

Puromycin is used in cell biology as a selective agent in cell culture systems. It is toxic to prokaryotic and eukaryotic cells. Resistance to puromycin is conferred by the pac gene encoding a puromycin N-acetyl-transferase (PAC). Cells transduced with plasmids that containing puromycin resistant gene will survive in Puromycin selection, which is widely used in stable cell line generation.

Specification:

1) 0.22ul filter sterilized solution in PBS at 5.0 mg/ml stock. Cell culture tested.
2) Compound name: Puromycin dihydrochloride
3) CAS number: 58-58-2
4) Cell-culture tested: toxicity and potency validated mammalian cell lines.
5) Formula: $C_{22}H_{29}N_7O_5 \cdot 2HCl$
6) Molecular weight: 544.43
7) Safety consideration: this solution is provided for stable cell selection, and for research use only, not for drug use. Please refer to its MSDS file for handling instructions.
8) Structure:

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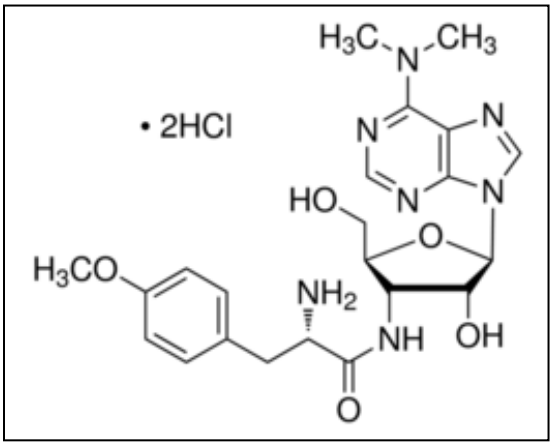

Working Concentrations:

The working concentration of antibiotic is dependent cell types and antibiotic's potency. It must be obtained from the killing curve test in your specific cell type. The optimal concentration is the minimal concentration that kill all your cells in the define time-course.

Puromycin kills eukaryotic cells very fast at low concentration compared to other antibiotics. The recommended Puromycin working concentration ranges from 0.1–5.0 μg/mL. Puromycin-resistant stable mammalian cell lines can be generated in one week.

The working concentration of Gentarget's Puromycin solution were tested in many mammalian cell types against Gentarget's lentivirus products containing corresponding antibiotic.

You can use the Puromycin's working concentration listed below for the matched cell type.

Working Concentration for Stable Cell Selection

Cell Line	**Puromycin** (CAT#: **P-anti**)
786-O cells (Human)	1
A549 (Human)	0.6

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ASPC1 (Human)	0.5
CFPAC-1 (Human)	1
CHO	20
CL-11 (Human)	5
CT26 (Mouse)	10
ES (embryonic stem)-2 (Human)	0.5
H1299 (Human)	1
HaCat (Human)	0.5
HCT116 (Human)	0.5
HEK293 (Human)	0.5
HeLa (Human)	0.6
Hep G2 (Human)	0.5
HL60S (Human)	0.5
Hsultans (Human)	1
Human HT29	0.5
HT1080 (Human)	0.2
HT22 (mouse)	1.2
Jurkat (Human)	0.5
MIA-PaCa-2	0.3
MB49 (Mouse)	0.3
MCF10A cell (Human)	0.5
MCF-7 (Human)	0.5
MDA-MB-231 (Human)	1
MOPC315 (Mouse)	5
Human MP41	0.5

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NCI-H1299 (Human)	1
Mouse Panc02	1.0
MP41 (Human)	0.5
panc-1 (Human)	1
PC3 (Human)	0.5
RAW 264.7 (Mouse)	2.5
RKO (Human)	0.5
RPMI8266 (Human)	0.6
SH-Sy5y (Human)	1.0
SHP-77 (Human)	1.0
SK-MEL-5 (Human)	0.3
SW1990 (Human)	0.5
SW403 (Human)	2.5 ~ 5.0
T47D cell (Human)	1
ZR-75-1 (Human)	0.6
U2-OS (Human)	0.6

*: Note: The working concentration above is provided as reference. You may test the killing curve for your cell culture conditions using following protocol.

Antibiotic Selection protocol:

Day 0:

Seed cells in complete medium at the appropriate density and incubate overnight in 24 well/plate.

Note: at the time of selection, cells should be 50%-75% confluent.

Day 1:

- Thaw Puromycin solution at 37°C. Under biological hood (sterilized condition), mix via pipetting to make sure it is in fully clear solution, no pellet left.

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- Make series dilution of Puromycin from 0.1 ug/ml to 5 ug/ml final concentration in your complemented medium.
- Remove the culture medium from the cell wells, and add 0.5ml puromycin containing medium (with the series diluted concentration) at each well.
- Return cells to grow in incubator.

Day 5:

Observe the cell death (detached or shirked) in each well. The optimal concentration is the minimal concentration that kill all cells in that well.

Then, use this optimal concentration to select your Puromycin-resistant stable cells in target wells where a puromycin resistant plasmid was transfected or transduced. Remember to set up the controls without transduction where all cells should be died after the selection.

Safety Precaution:

This antibiotic solution is provided for research use only, not for drug use, or clinical use. Please refer to MSDS file for handling this harmful material.