

# Product Sheet

## Puromycin Dihydrochloride

Version 3.0.1./260224

### Product description

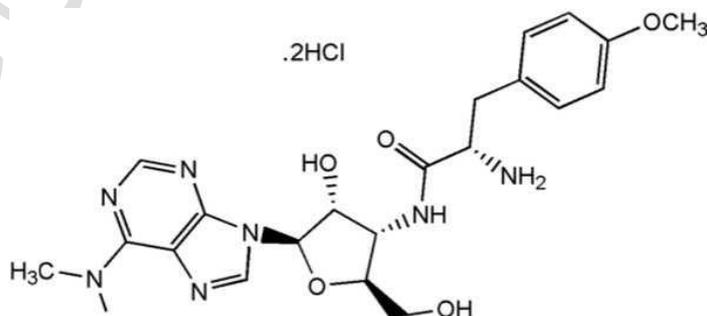
Puromycin is an aminonucleoside antibiotic produced by *Streptomyces alboniger*. It inhibits protein synthesis and is toxic to Gram-positive bacteria as well as a wide range of animal and insect cells; under certain conditions it can also affect *E. coli*. Puromycin acts as an analog of the 3' end of aminoacyl-tRNA. It enters the ribosomal A site and becomes incorporated into the elongating peptide chain. Because it does not support subsequent elongation reactions, it causes premature chain termination and releases immature polypeptides with puromycin at the C-terminus.

The **pac** gene found in *Streptomyces alboniger* encodes puromycin N-acetyltransferase (PAC), which confers resistance to puromycin. This property is widely used for selecting mammalian cells stably transfected with plasmids carrying the **pac** gene. Puromycin selection is also commonly used with lentiviral vectors, many of which include the **pac** gene. In certain cases, puromycin can be used to select *E. coli* transformants carrying **pac**-containing plasmids.

### Specifications

<b>Cat. No.</b>	GM-040401-1 / GM-040401-2 / GM-040401-3 / GM-040401-4 / GM-040401-5
<b>Packaging</b>	25 mg; 100 mg; 250 mg; 500 mg; 1 g
<b>CAS</b>	58-58-2
<b>Molecular Formula</b>	C <sub>22</sub> H <sub>29</sub> N <sub>7</sub> O <sub>5</sub> ·2HCl
<b>Molecular Weight</b>	544.43
<b>Purity</b>	>95% (HPLC)
<b>Concentration</b>	Up to 50 mg/mL in water
<b>Form</b>	Powder

### Structure



**Storage Conditions** Ship with ice packs. Store dry at -25°C to -15°C. Shelf life: 2 years.

## Usage Instructions

### 1 Recommended Concentrations

- **Mammalian cells:** 1–10 µg/mL. Determine the optimal concentration using a kill curve.
- **E. coli:** Select stable *E. coli* transformants carrying **pac** on LB agar at **125 µg/mL**.  
**Note:** Puromycin selection in *E. coli* requires precise pH adjustment and is affected by the host strain. After dissolution, sterilize by filtration using a **0.22 µm** filter.

### 2 Establishing a Puromycin Kill Curve (example: shRNA transfection or lentiviral transduction)

Effective selection concentration depends on cell type, growth status, density, metabolic state, and cell-cycle stage. To obtain stable shRNA-expressing cell lines, it is critical to determine the lowest puromycin concentration that kills non-transfected/non-transduced cells. A kill curve is strongly recommended for first-time use.

- 2.1 **Day 1:** Seed cells in a 24-well plate at **5–8 × 10<sup>4</sup> cells/well**. Prepare enough wells for a concentration gradient. Incubate overnight at 37°C.
- 2.2 **Day 2:**
  - (a) Prepare fresh selection media containing different puromycin concentrations (e.g., **0–15 µg/mL**, at least 5 gradients).
  - (b) Replace the medium with the prepared selection media. Incubate at 37°C.
- 2.3 **Day 4:** Replace with fresh selection media and observe cell viability.
- 2.4 Replace with fresh selection media every **2–3 days**, depending on cell growth.
- 2.5 Monitor cells daily and determine the minimum puromycin concentration that kills all non-transfected/non-transduced cells within **4–6 days** of starting selection.

### 3 Selection of Stable Mammalian Transfectants

After transfection with a **pac**-containing plasmid, cells are cultured in medium containing puromycin to select stable transfectants.

- 3.1 **48 h post-transfection**, culture cells (undiluted or diluted) in fresh medium containing an appropriate puromycin concentration.  
**Note:** Antibiotics work best when cells are actively dividing. High cell density reduces selection efficiency; keep confluence ≤25% when plating.
- 3.2 Replace the puromycin-containing medium every **2–3 days**.
- 3.3 After **7 days**, evaluate foci/colonies. Depending on the host cell line and selection efficiency, colony formation may require an additional week or longer.  
**Note:** Observe cell status daily. Puromycin selection requires at least **48 h**; typical selection duration at an effective concentration is **3–10 days**.
- 3.4 Transfer **5–10** resistant clones to **35 mm** dishes and maintain in selection medium for **7 days** to enrich cells for subsequent cytotoxicity assays.

## Notes

1. Puromycin is toxic; handle with care.
2. Puromycin has relatively low activity in *E. coli* but is highly effective in mammalian cells; it can be used as an alternative to the neomycin selection system in transfection experiments.
3. For research use only.