Genomeditech (Shanghai) Co., Ltd.
Order: +86 021-50432825/50432826
Email: service@genomeditech.com

Product Sheet

Puromycin Dihydrochloride

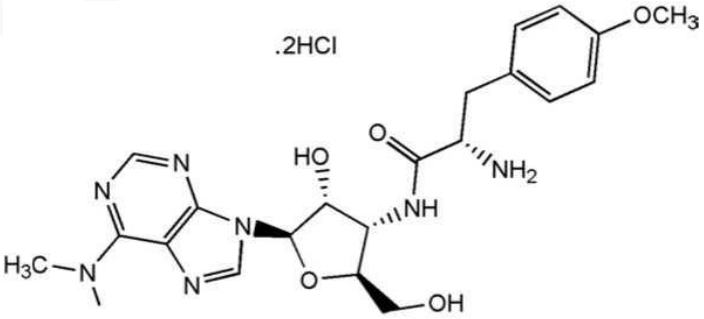
Version 3.0.1./260224

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

Cat. No.	GM-040401-1 / GM-040401-2 / GM-040401-3 / GM-040401-4 / GM-040401-5
Packaging	25 mg; 100 mg; 250 mg; 500 mg; 1 g
CAS	58-58-2
Molecular Formula	$C_{22}H_{29}N_7O_5 \cdot 2HCl$
Molecular Weight	544.43
Purity	>95% (HPLC)
Concentration	Up to 50 mg/mL in water
Form	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 × 10^4 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.