



BacOut™ Antibiotics

Cat. No. G7000

Store at -20 °C (stable for 12 months) **OR** at 4°C for daily use (stable for 2 months).

Product Description

Bacterial contamination remains one of the most critical challenges in daily cell culture work. Once a culture becomes contaminated, the medium turns turbid, often forcing researchers to discard valuable cells. In situations where no backup inventory exists — such as during long-term or labor-intensive projects like **hybridoma clone screening** — the resulting loss in time, resources, and effort can be immeasurable.

To address this common but costly issue, **abm** has developed **BacOut™ Antibiotics** – a proprietary **broad-spectrum antibiotic set** designed specifically for cell culture rescue. Unlike standard antibiotics, BacOut™ is tailored for both **routine contamination prevention** and **specialized recovery services**, helping you preserve and protect your most precious cell lines.

The 3 antibiotics included in this product set **cannot be premixed**, as precipitation will occur. Each of the antibiotics has to be added separately to your contaminated culture to avoid possible precipitation.

Key Features:

- **Broad-spectrum protection** against common bacterial contaminants in cell culture
- **Dual utility:** available as a **ready-to-use product** and as a part of **abm's cell rescue services**
- **Safeguards valuable research projects** (e.g.: hybridoma clone screening) by reducing the risk of losing irreplaceable cell lines
- **Convenient storage** at -20 °C for up to 12 months **or** at 4 °C for 2 months (if using daily)

Cat. No.	Description	Quantity
G7000-A	BacOut™ Antibiotic Reagent - A	2 ml
G7000-B	BacOut™ Antibiotic Reagent - B	2 ml
G7000-C	BacOut™ Antibiotic Reagent - C	2 ml

BacOut™ Cell Culture Decontamination Protocol

1. Initial Wash – Reduce Free-Floating Bacteria

Before starting treatment, wash cells thoroughly to minimize the existing bacterial load:

- **Adherent cells:** Rinse vigorously with **serum-free medium** or **1× PBS** directly in the flask or culture plate.
- **Suspension cells:** Perform **multiple centrifugations and wash cycles** using culture medium or **1× PBS**.

*Tip: For best results, perform treatment in a **T25 or T75 flask with a vented cap** to ensure ease of handling and optimal gas exchange.*

2. Initiate BacOut™ Treatment

- Begin treatment when cells are at **80–90% confluency** (high density preferred).
- Add **BacOut™** to the culture medium at a **1:50 dilution** for **each antibiotic** included in the set.
 - *Example: Add **100 µL BacOut™** to **5 mL culture medium** for each of the three antibiotics provided.*

△ **Note:** The three antibiotics in the BacOut™ set **must not be premixed**, as precipitation will occur if combined prior to use.

*Tip: Start treatment **early in the day (ideally 8:00–9:00 a.m.)**. The first day is critical for successful contamination control since bacteria can replicate every ~20 minutes.*

3. Frequent Treatment Cycles (Day 1)

- On day 1: Repeat **Steps 1 and 2 every 1.5–2 hours**.
- Gently **swirl the culture medium** in the flask to ensure BacOut™ contacts all surfaces, including the upper areas of the culture vessel (T-flasks are recommended).
- Aim for **a total of 4 treatment cycles** on Day 1.

4. Assess Cytotoxicity (End of Day 1, 4:00-5:00 p.m.)

- Examine cultures under a microscope.
 - If **no significant cytotoxicity** is observed → continue overnight incubation treatment with BacOut™ at **1:50 dilution**.
 - If **cytotoxic effects are observed** → reduce BacOut™ concentration to **1:100** for overnight incubation.

5. Day 2 and Beyond

- By Day 2, bacterial growth should be under control.
- Continue treatment:
 - Repeat treatment (Step 2) using BacOut™ at a **1:100 dilution** once a day.
 - Maintain this routine for an additional **2 days**.

6. Recovery Phase

- Transition cultures back to long-term conditions by supplementing the medium with **BacOut™ at 1:200 dilution** for an additional **2 weeks**.

*Tip: Prepare and store a **backup vial of recovered cells** before resuming standard antibiotic conditions.*

7. Criteria for Successful Decontamination

- Cells maintain growth for **≥2 weeks** under standard antibiotic conditions (or no antibiotics) **without recurrence of contamination, OR**
- Confirmation of absence of bacteria by **PCR or Next-Generation Sequencing (16S rRNA detection)**.

General Notes

- Strict adherence to the **Day 1 treatment schedule** (frequent interventions) is essential to maximize bacterial clearance while preserving cell viability.