

Antibiotic and Selection Reagent Usage for Resistant *E. coli* Cells

ATCC No.	Stock Solutions			Working Concentrations
	Antibiotic	Concentration	Storage Temp.	
60-2000	Ampicillin	10 mg/ml in water	-20°C	20 to 125 µg/ml
60-2003	Chloramphenicol	10 mg/ml in ethanol	-20°C	25 to 170 µg/ml
60-2001	Kanamycin	10 mg/ml in water	-20°C	10 to 50 µg/ml
60-2002	Tetracycline	5 to 10 mg/ml in 50% ethanol	-20°C	10 to 50 µg/ml
60-2004	Carbenicillin	5 mg/ml in water	-20°C	50 to 100 µg/ml
30-2303	Gentamicin	10 to 50 mg/ml in water	-20°C	5 to 20 µg/ml
30-2305	G418	10 to 50 mg/ml in water or buffered solution	-20°C	Bacterial: 10 to 50 µg/ml Mammalian: 200 µg/ml for maintenance, ~400 µg/ml for selection Yeast: 0.5 to 1.0 mg/ml
60-2005	X-Gal	20 mg/ml in dimethylformamide	-20°C	40 to 50 µl/ml or 40 µl of stock solution spread onto surface of a 100-mm agar plate
60-2006	IPTG	100 mM	-20°C	0.01 mM or 40 µl of stock solution spread onto surface of a 100-mm agar plate

- Store all antibiotic stock solutions in the dark at -20°C.
- Tetracycline must always be wrapped in foil and stored in the dark whether it is in stock solution, in broth, or in agar.
- Stock solutions in water must be filter sterilized through a 0.22-µm filter. Antibiotics dissolved in ethanol need not be sterilized.
- Do not use expired antibiotics to make stock solutions, as they may be labile and may result in inconsistent results.
- Add antibiotics to liquid medium immediately prior to use from properly stored sterile stock solutions.
- Lower concentrations are suitable for the selection of cells containing low copy number plasmids, higher concentrations for high copy number plasmids.
- Agar medium should be cooled to 50°C or lower before the addition of heat-sensitive antibiotics and nutrients.
- Plates should be stored upside down at 4°C in the dark (foil wrapped) for no longer than three months due to degradation of antibiotics.
- Magnesium is an antagonist to tetracycline; use media without magnesium salts when working with bacteria that are tetracycline resistant.

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