

# Hygromycin B Protocol and Selection Guide

## Background

Hygromycin B is an aminoglycoside antibiotic which targets both prokaryotic and eukaryotic cells. Hygromycin B is freely soluble in water (50 mg/mL). Hygromycin B can be used to select for a resistance plasmid in numerous cell lines. Effective working concentrations range from 100 – 1000 µg/mL

## Preparation and storage

Hygromycin B is soluble in water at >50 mg/mL. It is also soluble in methanol or ethanol. Solutions should be sterilized by filtration, not by autoclaving.

Hygromycin B solutions have been reported to lose activity on freezing. Since solutions are stable refrigerated, freezing should be avoided. Hygromycin B products should be stored as supplied at 2-8 °C. The dry solid is stable for at least five years if stored at 2-8 °C. Hygromycin B solutions are stable as supplied for two years if stored at 2-8 °C.

## Mammalian Cell Culture

### Kill curve procedure

To express a recombinant protein of interest in mammalian cells, a mammalian expression vector containing the hyg or hph gene must be introduced into the mammalian host cell line. The hyg or hph marker gene allows for selection of stable transfectants using hygromycin B resistance.

To generate a stable cell line expressing your protein of interest, you need to determine the minimum concentration of antibiotic required to kill your untransfected host cell line. Test a range of concentrations at least to ensure that you determine the minimum concentration necessary for your cell line.

1. Dilute cells in culture medium to  $2.0 \times 10^4$  cells/ml. Add 4.0 ml of the cell dilution to each well of ten 6-well plates. For cells that require higher densities for viability, proportionally decrease the cell dilution. Allow cells to adhere overnight.
2. Prepare a 5.0 mg/ml solution of hygromycin B in distilled deionized water if the antibiotic is in powder form. If the antibiotic is in liquid form, make a 1:200 dilution in distilled deionized water. Make test dilutions of hygromycin B using the following dilution chart:

Final Concentration	Culture Medium	Hygromycin B Solution (5.0 mg/ml)
0.05 mg/ml	29.7 ml	0.30 ml
0.10 mg/ml	29.4 ml	0.60 ml
0.25 mg/ml	28.5 ml	1.50 ml
0.50 mg/ml	27.0 ml	3.00 ml
1.00 mg/ml	24.0 ml	6.0 ml

3. Substitute culture medium with medium containing the varying concentrations of hygromycin B.

4. Count the number of viable cells at regular intervals to determine the appropriate concentration of antibiotic that prevents growth of untransfected cells. Select the concentration that kills the majority of the cells in the desired number of days, generally 7-10 days.

### General Selection Table

Cell-Line	Species	Tissue	Media	Hygromycin B
293	Human	Kidney; fetal	DMEM	200 (µg/ml)
293	Human	Kidney; fetal	Eagle's minimal essential medium	200 (µg/ml)
293T	Human	Kidney (Embryonic)	DMEM	250 (µg/ml)
3T3	Mouse	Embryonic fibroblast	DMEM	150 (µg/ml)
3T3-L1	Mouse	Embryo	DMEM	110 (µg/ml)
A-431	Human	Epidermis; epidermoid carcinoma	DMEM	200 (µg/ml)
A549	Human	Lungcarcinoma	RPMI	300 (µg/ml)
A9	Mouse/Mouse	Hybridoma	DMEM	100 (µg/ml)
A9	Mouse/Mouse	Hybridoma	DMEM	200 (µg/ml)
AtT-20	Mouse	Pituitary tumor	DMEM	200 (µg/ml)
AtT-20	Mouse	Pituitary tumor	DMEM	400 (µg/ml)
AtT-20/D16v-F2	Mouse	Pituitary tumor	DMEM	300 (µg/ml)
BALB/3T3 clone A31	Mouse	Embryo	DMEM	500 (µg/ml)
BALB/3T3 clone A31	Mouse	Embryo	DMEM	200 (µg/ml)
BD EcoPack2-293			DMEM	100 (µg/ml)
BHK-21 (C-13)	Hamster, Syrian golden	Kidney	Eagle's minimal essential medium	80 (µg/ml)
BRL 3A	Rat	Liver	DMEM	100 (µg/ml)
C2C12	Mouse	Muscle	DMEM	400 (µg/ml)
C2C12	Mouse	Muscle	DMEM	150 (µg/ml)
Caco-2	Human	Colon; colorectal adenocarcinoma	DMEM	175 (µg/ml)
CHO	Hamster, Chinese	Ovary	Ham's F-12 nutrient mixture	150 (µg/ml)
CHO-K1	Hamster, Chinese	Ovary	DMEM	150 (µg/ml)
CHO-K1	Hamster, Chinese	Ovary	Ham's F-12 nutrient mixture	250 (µg/ml)

COLO 320DM	Human	Colon; colorectal adenocarcinoma	DMEM	400 (µg/ml)
COS-7	Monkey, African green	Kidney	DMEM	400 (µg/ml)
COS-7	Monkey, African green	Kidney	DMEM	200 (µg/ml)
F9	Mouse	Testis; Embryonic carcinoma; testicular teRatoma	DMEM	600 (units/ml)
G355-5	cat	Brain; astrocyte	DMEM	150 (µg/ml)
G-401	Human	Kidney; rhabdoid tumor	DMEM	400 (µg/ml)
GH3	Rat	Pituitary tumor	DMEM	500 (µg/ml)
HCC1937	Human	mammary gland; primary ductal carcinoma	RPMI	200 (µg/ml)
HCT 116	Human	Colon; colorectal carcinoma	DMEM	350 (µg/ml)
HCT 116	Human	Colon; colorectal carcinoma	DMEM	200 (µg/ml)
HeLa S3	Human	Cervix; adenocarcinoma	DMEM	600 (µg/ml)
Hep G2	Human	Liver; hepatocellular carcinoma	DMEM	200 (µg/ml)
HEp-2	Human	HeLa contaminant	DMEM	60 (µg/ml)
JAR	Human	placenta; choriocarcinoma	RPMI	150 (µg/ml)
JAR	Human	placenta; choriocarcinoma	DMEM	200 (µg/ml)
LNCaP	Human	prostatic adenocarcinoma	RPMI	200 (µg/ml)
LS 174T	Human	Colon; colorectal adenocarcinoma	RPMI	50 (µg/ml)
MDA-MB-435	Human	breast	Minimal essential medium	200 (µg/ml)
Neuro-2a	Mouse	Brain; neuroblast; neuroblastoma	DMEM	400 (µg/ml)
Neuro-2a	Mouse	Brain; neuroblast; neuroblastoma	DMEM	200 (µg/ml)
NIH/3T3	Mouse	Embryo	DMEM	200 (µg/ml)
NIH/3T3	Mouse	Embryo	DMEM	150 (IU/ml)
NIH/3T3	Mouse	Embryo	DMEM	400 (µg/ml)
NIH3T3	Mouse	Embryo	DMEM	100 (µg/ml)
NIH3T3	Mouse	Embryo		1 (µg/ml)
NRK	Rat	Kidney		260 (µg/ml)
PA317	Mouse	Embryo; amphotropic retroviral packaging line	RPMI	100 (µg/ml)
PA317	Mouse	Embryo; amphotropic retroviral packaging line	DMEM	400 (µg/ml)
PANC-1	Human	Pancreas (duct); epithelioid carcinoma	DMEM	40 (µg/ml)
PANC-1	Human	Pancreas (duct); epithelioid carcinoma	Minimal essential medium	200 (µg/ml)
Rat2	Rat	Fetus	DMEM	800 (µg/ml)
SH-SY5Y	Human	Bone marrow (metastasis); neuroblastoma (Brain primary)		400 (µg/ml)
SK-BR-3	Human	pleural effusion (metastasis); adenocarcinoma (Colon primary)	DMEM	100 (µg/ml)

T-47D	Human	pleural effusion (metastasis); ductal carcinoma (mammary gland primary)	DMEM / F 12 medium	300 (µg/ml)
U-2 OS	Human	Bone; osteosarcoma	DMEM	100 (µg/ml)
U-937	Human	macrophage; histiocytic lymphoma	RPMI	200 (µg/ml)

## References:

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[http://www.mpbio.com/detailed\\_info.php?family\\_key=02157513](http://www.mpbio.com/detailed_info.php?family_key=02157513)

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