

G418 Protocol and Selection Guide

Background

G418 is routinely used in gene selections to screen for resistant mammalian cells which express the neo gene. The neo gene encodes amino-glycoside 3'-phosphotransferase; an enzyme which renders the cell resistant to G418. For gene selection applications, a kill curve can be made to determine the minimum effective G418 concentration to kill non-resistant cells.

Preparation and storage

Generally it is easiest to dissolve the entire container of G418 at once (especially if it is a small 1g container).

- Calculate the amount of PBS to add to the vial to get a 200mg/ml 'active' concentration.
 - For example: 710µg/mg means that 710µg out of 1000µg are active, or that the Geneticin is 71% active. Therefore, to make a stock solution of 200mg/ml active Geneticin you need to add $1000/710 \times 200 = 281.6901$ mg/ml of Geneticin. The vial contains 1g of Geneticin, so you need to dissolve the contents of the vial in $1000\text{mg}/281.6901\text{mg} = 3.55$ ml total volume.
- Sterile filter the drug using a syringe and a 0.45µm filter.
- Store stock at -80°C and working stock solution at 4°C

Mammalian Cell Culture

Selection procedure for mammalian cells

G418 sulfate is normally used at a concentration of 400 µg/ml. After transfection with a plasmid containing the *neo* gene, cells are incubated in their regular growth medium containing G418 to select for stable transfectants.

1. 48 hours post-transfection, pass cells (direct or diluted) in fresh medium containing G418 at the appropriate concentration.
 - a) *Note: Antibiotics work best when cells are actively dividing. If the cells become too dense, the antibiotic efficiency will decrease. It is best to split cells such that they are not more than 25% confluent.*
2. Remove and replace antibiotic containing medium every 3-4 days
3. Evaluate cells for the formation of foci after 7 days of selection. Foci may require an additional week or more to develop depending on the host cell line and transfection/selection efficiency.

4. Transfer and pool 5-10 resistant clones to a 35mm cell culture plate and maintain on selection medium for an additional 7 days. This pooled culture will be expanded for subsequent cytotoxicity assays.

General Selection Table

Cell line	Species	Tissue	Culture medium	G418
293	Human	Kidney; fetal	DMEM	600 (µg/ml)
293	Human	Kidney; fetal	Minimal essential medium	800 (µg/ml)
143B	Human	bone; osteosarcoma	DMEM	400 (µg/ml)
293T	Human	Kidney (embryonic)	DMEM	1000 (µg/ml)
32D Clone 3	Mouse	bone marrow	RPMI	600 (µg/ml)
3T3	Mouse	embryonic fibroblast	DMEM	1000 (µg/ml)
3T3-L1	Mouse	embryo	DMEM	1500 (µg/ml)
70Z/3	Mouse	pre-B lymphoblast; methylnitrosourea-induced lymphoma	DMEM	400 (µg/ml)
A20	Mouse	B lymphocyte; reticulum cell sarcoma	DMEM	1000 (µg/ml)
A20	Mouse	B lymphocyte; reticulum cell sarcoma	RPMI	400 (µg/ml)
A-204	Human	muscle; rhabdomyosarcoma	DMEM	500 (µg/ml)
A253	Human	epidermis; epidermoid carcinoma	RPMI	800 (µg/ml)
A-431	Human	epidermis; epidermoid carcinoma	α-modified minimal essential medium	800 (µg/ml)
A431NS	Human	epidermis; epidermoid carcinoma	DMEM	800 (µg/ml)
A-498	Human	Kidney; carcinoma	DMEM	1000 (µg/ml)
A549	Human	Lungcarcinoma	RPMI	700 (µg/ml)
A9	Mouse/Mouse	hybridoma	DMEM	500 (µg/ml)
AGS	Human	stomach; gastric adenocarcinoma	RPMI	400 (µg/ml)
AR42J	Rat	exocrine pancreas; tumor	DMEM	800 (µg/ml)
AtT-20	Mouse	pituitary tumor	DMEM	600 (µg/ml)
BALB/3T3 clone A31	Mouse	embryo	DMEM	500 (µg/ml)
BeWo	Human	placenta; choriocarcinoma	Ham's F-12 nutrient mixture	500 (µg/ml)
BHK-21 (C-13)	Hamster, Syrian golden	Kidney	DMEM	1000 (µg/ml)
Bing	Human	Kidney; amphotropic retroviral packaging line	DMEM	800 (µg/ml)
BJ	Human	skin (foreskin)	DMEM	400 (µg/ml)
BRL 3A	Rat	liver	DMEM	200 (µg/ml)
BT	bovine	turbinata	DMEM	400 (µg/ml)
BT-20	Human	mammary gland; carcinoma	Eagle's minimal essential medium	250 (µg/ml)
BT-474	Human	mammary gland; ductal carcinoma	DMEM	200 (µg/ml)
BT-549	Human	mammary gland; ductal carcinoma	DMEM	750 (µg/ml)
C166	Mouse (transgenic)	yolk sac; endothelial cell differentiation model; stem cell feeder layer	Minimal essential medium	800 (µg/ml)

C2C12	Mouse	muscle	DMEM	4000 (µg/ml)
C2C12	Mouse	muscle	DMEM	500 (µg/ml)
C3H10T1/2	Mouse	embryonic mesenchymal cell line	DMEM	500 (µg/ml)
C6	Rat	brain; glioma	DMEM	1000 (µg/ml)
Caco-2	Human	colon; colorectal adenocarcinoma	DMEM	500 (µg/ml)
Caco-2	Human	colon; colorectal adenocarcinoma	Minimal essential medium	1 (mg/ml)
Chang Liver	Human	HeLa contaminant	DMEM	350 (µg/ml)
CHO	Hamster, Chinese	ovary	Ham's F-12 nutrient mixture	1000 (µg/ml)
CHO-K1	Hamster, Chinese	ovary	DMEM	600 (µg/ml)
CHO-K1	Hamster, Chinese	ovary	DMEM / F 12 medium	150 (µg/ml)
CHO-K1	Hamster, Chinese	ovary	DMEM + F12 medium	800 (µg/ml)
CHO-K1	Hamster, Chinese	ovary	Eagle's minimal essential medium	500 (µg/ml)
CHO-K1	Hamster, Chinese	ovary	Ham's F-12 nutrient mixture	1000 (µg/ml)
CHO-K1	Hamster, Chinese	ovary	Minimal essential medium	800 (µg/ml)
CHO-K1	Hamster, Chinese	ovary	α-modified minimal essential medium	400 (µg/ml)
Clone 9	Rat	liver	DMEM / F 12 medium	600 (µg/ml)
COLO 320DM	Human	colon; colorectal adenocarcinoma	DMEM	300 (µg/ml)
COLO 320HSR	Human	colon; colorectal adenocarcinoma	DMEM	400 (µg/ml)
COS-1	monkey, African green	Kidney	DMEM	1000 (µg/ml)
COS-7	monkey, African green	Kidney	RPMI	500 (µg/ml)
CRFK	cat	Kidney (cortex)	DMEM	2000 (µg/ml)
CRFK	cat	Kidney (cortex)	RPMI	500 (µg/ml)
CSM14.1				2000 (µg/ml)
CV-1	monkey, African green	Kidney	DMEM	800 (µg/ml)
D17	dog	bone; osteosarcoma	RPMI	600 (µg/ml)
DLD-1	Human	colon; colorectal adenocarcinoma	DMEM	600 (µg/ml)
DLD-1	Human	colon; colorectal adenocarcinoma	RPMI	1000 (µg/ml)
DLD-1	Human	colon; colorectal adenocarcinoma	RPMI	400 (µg/ml)
DO11.10				500 (µg/ml)
DT40	chicken	bursa; lymphoma	RPMI	1000 (µg/ml)
DT40	chicken	bursa; lymphoma	RPMI	350 (µg/ml)
DT40	chicken	bursa; lymphoma	RPMI	500 (µg/ml)
DU 145	Human	brain (metastasis); carcinoma (prostate primary)	DMEM	200 (µg/ml)
E11 (HCT-8 variant)	Human	colon	RPMI	800 (µg/ml)

ES-E14TG2a	Mouse	embryonic stem cell; pluripotent; HGRPT deficient		200 (µg/ml)
FRTL	Rat	thyroid		600 (µg/ml)
GH3	Rat	pituitary tumor	DMEM	1 (µg/ml)
GH4	Rat		DMEM	800 (µg/ml)
GLC-82	Human	lung	RPMI	500 (µg/ml)
GP+E-86	Mouse	embryo; amphotropic retroviral packaging line	DMEM	400 (µg/ml)
GP+envAM-12	Mouse	embryo; amphotropic retroviral packaging line	DMEM	800 (µg/ml)
H1299	Human	lung	RPMI	500 (µg/ml)
HeLa	Human	cervix; adenocarcinoma	DMEM	1000 (µg/ml)
HeLa	Human	cervix; adenocarcinoma	DMEM + F12 medium	600 (µg/ml)
HeLa	Human	cervix; adenocarcinoma	Eagle's minimal essential medium	400 (µg/ml)
HeLa S3	Human	cervix; adenocarcinoma	DMEM	300 (µg/ml)
HeLa S3	Human	cervix; adenocarcinoma	Ham's F-12 nutrient mixture	700 (µg/ml)
Hep 3B2.1-7	Human	liver; hepatocellular carcinoma	Eagle's minimal essential medium	2000 (µg/ml)
Hep 3B2.1-7	Human	liver; hepatocellular carcinoma	Minimal essential medium	2 (mg/ml)
Hep 3B2.1-7	Human	liver; hepatocellular carcinoma	Minimal essential medium	500 (µg/ml)
Hep G2	Human	liver; hepatocellular carcinoma	DMEM	700 (µg/ml)
HEp-2	Human	HeLa contaminant	DMEM	1000 (µg/ml)
Hs27	Human	skin (foreskin)	DMEM	850 (µg/ml)
HT-1080	Human	connective tissue; fibrosarcoma	DMEM	250 (µg/ml)
HuT 78	Human	T lymphocyte; cutaneous; lymphoma	DMEM	1000 (µg/ml)
JAR	Human	placenta; choriocarcinoma		1000 (µg/ml)
JB6 Cl 41-5a	Mouse	skin (epidermis); chemically transformed	Eagle's minimal essential medium	800 (µg/ml)
JEG-3	Human	placenta; choriocarcinoma	DMEM	500 (µg/ml)
Jurkat, Clone E6-1	Human	T lymphocyte; acute T cell leukemia		750 (µg/ml)
K562	Human	lymphoblastoid	RPMI	400 (µg/ml)
K-562	Human	pleural effusion (metastatic); chronic myelo-genous leukemia (bone marrow primary)	DMEM	850 (µg/ml)
KB	Human	HeLa contaminant	DMEM	1000 (µg/ml)
LLC-PK1	pig	Kidney	Charcoal-stripped fetal bovine serum	1500 (µg/ml)
LMH	chicken	liver; hepatocellular carcinoma	DMEM / F 12 medium	800 (µg/ml)
LNCaP	Human	prostatic adenocarcinoma	RPMI	1500 (µg/ml)
LS 174T	Human	colon; colorectal adenocarcinoma	RPMI	800 (µg/ml)
M1	Mouse	myeloblast; myeloid leukemia	DMEM	100 (µg/ml)
M1	Mouse	myeloblast; myeloid leukemia		600 (µg/ml)
MC3T3-E1	Mouse	bone (metastasis); osteosarcoma	DMEM	500 (µg/ml)

Subclone 4		(femur primary)		
MCF 10A	Human	mammary gland; fibrocystic disease	DMEM	1000 (µg/ml)
MCF-12A	Human	mammary gland	DMEM	600 (µg/ml)
MCF7	Human	pleural effusion (metastasis); adenocarcinoma (mammary gland primary)		800 (mg/ml)
MDA-MB-231	Human	pleural effusion (metastasis); adenocarcinoma (mammary gland primary)	DMEM	700 (µg/ml)
MDA-MB-468	Human	pleural effusion (metastasis); adenocarcinoma (mammary gland primary)	Minimal essential medium	2 (µg/ml)
MDCK (NBL-2)	dog	Kidney	DMEM	400 (µg/ml)
ME-180	Human	omentum (metastasis); epidermoid carcinoma (cervix primary)	RPMI	1000 (µg/ml)
MG-63	Human	bone; osteosarcoma	DMEM	400 (µg/ml)
MV-4-11	Human	peripheral blood; biphenotypic B myelomonocytic leukemia	RPMI	400 (µg/ml)
NCI-H1299	Human	lymph node (metastasis); large cell neuroendocrine carcinoma (lung primary)	DMEM	700 (µg/ml)
Neuro-2a	Mouse	brain; neuroblast; neuroblastoma	DMEM	1000 (µg/ml)
NG108-15	Mouse/Rat	brain; glial cell; neuron; glioblastoma; neuroblastoma (hybrid)	DMEM	1000-1300 (µg/ml)
NIH/3T3	Mouse	embryo	DMEM	1000 (µg/ml)
NIH3T3	Mouse	embryo	DMEM	100 (µg/ml)
NRK	Rat	Kidney	DMEM	200 (µg/ml)
NRK-52E	Rat	Kidney	DMEM	500 (µg/ml)
OK	opossum	Kidney (cortex); proximal tubule	DMEM + F12 medium	400 (µg/ml)
P19	Mouse	embryo; teRatocarcinoma; embryonic carcinoma	DMEM	500 (µg/ml)
PA317	Mouse	embryo; amphotropic retroviral packaging line	DMEM	1000 (µg/ml)
PA317	Mouse	embryo; amphotropic retroviral packaging line	DMEM	200 (µg/ml)
PA317	Mouse	embryo; amphotropic retroviral packaging line	DMEM	800 (µg/ml)
PANC-1	Human	pancreas (duct); epithelioid carcinoma	DMEM	800 (µg/ml)
PANC-1	Human	pancreas (duct); epithelioid carcinoma	Minimal essential medium	550 (µg/ml)
PC-12	Rat	adrenal gland; pheochromocytoma	DMEM	400 (µg/ml)
PC-12	Rat	adrenal gland; pheochromocytoma	DMEM	700 (µg/ml)
PC-3	Human	bone (metastasis); adenocarcinoma (prostate primary)	Ham's F-12 nutrient mixture	1000 (µg/ml)
PLC/PRF/5	Human	liver; Alexander cells; hepatoma	DMEM	400 (µg/ml)
PT67	Mouse	embryo; amphotropic retroviral packaging line	DMEM + F12 medium	800 (µg/ml)

PT67	Mouse	embryo; amphotropic retroviral packaging line		500 (µg/ml)
RD	Human	muscle; rhabdomyosarcoma	DMEM	100 (µg/ml)
Saos-2	Human	bone; osteosarcoma	DMEM	1 (mg/ml)
Saos-2	Human	bone; osteosarcoma	DMEM	300 (µg/ml)
Saos-2	Human	bone; osteosarcoma	DMEM	400 (µg/ml)
SC	Human	peripheral blood; macrophage; monocyte	DMEM	400 (µg/ml)
SCC-25	Human	tongue; squamous cell carcinoma	DMEM + F12 medium	400 (µg/ml)
Sf9	armyworm, fall	ovary	SF900 II serum free medium	350 (µg/ml)
SH-SY5Y	Human	bone marrow (metastasis); neuroblastoma (brain primary)	DMEM	500 (µg/ml)
SiHa	Human	cervix; squamous cell carcinoma	DMEM	800 (µg/ml)
SIRC (Statens Seruminstitut Rabbit Cornea)	rabbit	eye (cornea)	DMEM	600 (µg/ml)
SK-HEP-1	Human	ascites (metastasis); adenocarcinoma (liver primary)	DMEM	500 (µg/ml)
SK-N-MC	Human	supraorbital area (metastasis); neuroepithelioma (brain primary)	DMEM	1000 (µg/ml)
SK-N-SH	Human	bone marrow (metastasis); neuroblastoma (brain primary)	DMEM	1000 (µg/ml)
STO	Mouse	embryo	DMEM	800 (µg/ml)
SW 1353	Human	bone; chondrosarcoma	α-modified minimal essential medium	500 (µg/ml)
SW480	Human	colon; colorectal adenocarcinoma	DMEM	1000 (µg/ml)
T2 (174 x CEM.T2)	Human/Human	lymphoblast cell hybrid	RPMI	600 (µg/ml)
T24	Human	urinary bladder; transitional cell carcinoma	DMEM	50 (µg/ml)
T-47D	Human	pleural effusion (metastasis); ductal carcinoma (mammary gland primary)	RPMI	400 (µg/ml)
T-47D	Human	pleural effusion (metastasis); ductal carcinoma (mammary gland primary)	RPMI	800 (µg/ml)
U-2 OS	Human	bone; osteosarcoma	DMEM	700 (µg/ml)
U-2 OS	Human	bone; osteosarcoma	RPMI	600 (µg/ml)
U-937	Human	macrophage; histiocytic lymphoma	RPMI	500 (µg/ml)
U-937	Human	macrophage; histiocytic lymphoma	α-modified minimal essential medium	400 (µg/ml)
WEHI-231	Mouse	B lymphocyte; lymphoma	RPMI	600 (µg/ml)

References:

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