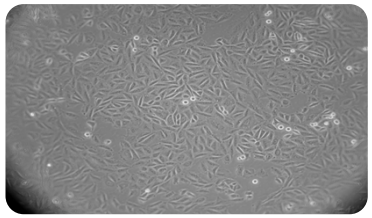
Cell Culture Protocol
CHO hERG DUO

Company: **BSYS**
Ion channel: **hERG**
MTA ID No.: **B.6.0**



Materials & Catalogue Numbers

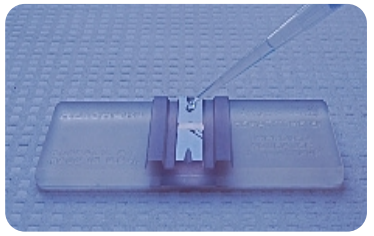
Reagents:	
PBS (without calcium and magnesium)	D8537 Sigma
Trypsin/EDTA	T4174 Sigma
Trypan blue	T8154 Sigma
T175 culture flasks	159910 Nunc
Serum-free Media:	
25 ml CHO-cell (SFM)	C5467 Sigma
25 mM Hepes	H0887 Sigma
100U/ml Penicillin/Streptomycin (P/S)	P4333 Sigma
0.04 mg/ml Soy bean trypsin inhibitor (SBTI)	T6522 Sigma
Culture Media:	
500 ml DMEM/F12 media	D6434 Sigma
10% Foetal Bovine serum (FBS)	F6178 Sigma
100µg/ml Geneticin (G418)	G8168 Sigma
100µg/ml Hygromycin B	H0654 Sigma
2mM L-Glutamin	G8541 Sigma

Culturing of cells from frozen vials

1. Thaw the vial quickly in a 37ºC water bath
2. Transfer the content of the vial to a T75 flask containing pre-heated **Culture Media**.
3. Change the **Culture Media** the next day.
4. Let the cells reach 70-80% confluence before **sub-culturing**. After 1-2 weeks of **sub-culturing** the cells have reached a stable growth pattern and will be suitable for QPatch experiments.

Sub-culturing (T175)

1. Remove old **Culture Media** and wash with 7 ml **PBS**.
2. Add **Trypsin**, gently swirl the flask and aspirate (leave about 1 ml)
3. Place the culture flask in 37ºC incubator for ~2 min. (ensure that the cells have a round shape before tapping).
4. Gently tap on the side of the flask and add 5-7 ml **Culture Media** and resuspend the cells by working the cell suspension up and down 5-10 times.
5. Determine the cell density and viability by counting the cells in a Hemocytometer using Trypan Blue.
6. Add the number of cells to the mother flask and the experiment flasks according to the **Sub-culturing Plan** below.
7. Grow the cells at 37ºC, 5% CO_2 to maximum 70-80% confluence.



Sub-culturing plan for making mother flasks and experiment flasks

1. Add $3x10^4$ cells/cm^2 for sub-culturing/experiments after 24 hours.
2. Add $1.6x10^4$ cells/cm^2 for sub-culturing/experiments after 48 hours.*
3. Add $8x10^3$ cells/cm^2 for sub-culturing/experiments after 72 hours.*
4. Add $6x10^3$ cells/cm^2 for sub-culturing/experiments after 96 hours.

For passage of CHO cells we recommend to **Sub-Culture** cells every Monday, Wednesday and Friday.
*We recommend 48 or 72 hours of sub-culturing for best results.

Cells for experiments (for T175)

1. Remove **Culture Media** and wash with 7 ml **PBS**.
2. Add 3 ml **Trypsin**, gently swirl the flask and aspirate (leave about 1 ml).
3. Place the culture flask in a 37ºC incubator for ~2 min (ensure that the cells have reached a round shape before tapping).
4. Add 5 ml **Serum-Free Media** and resuspend the cells by working the cell suspension up and down 5-10 times.
5. Determine the cell density and viability by diluting an aliquot 1:2 in **Trypan Blue** and count the cells in a Hemocytometer.
6. Make sure that there are 2-5 mill/ml cells added to the QStirrer