

CHO-Transfection to generate stable Flp-In expression cell-lines:

1: Determine the sensitivity of our CHO-Cell-Line to Hygromycin B: (is optional)

- Use variant concentrations of Hygromycin B (suggested range from Invitrogen is 500-600 µg/ml)

Material:

- CHO-stock
- 75 cm²-flask
- 6-wells
- Hygromycin B
- Complete-media, Trypsin/EDTA, PBS

Ca. 5-6 days

2: Cotransfection with Flp-In-Construct and pOG44:

Material:

- 6-wells mit CHO-Zellen (Medium ohne Zeocin)
- Lipofectamin oder **PEI** (Polyethylenimin)
- pOG44 und Konstrukt-Vektor
- **Opti-MEM** (medium für Transfection)
- 48 h nach Transfektion split the cells to max 25 % confluence in Media mit Hygromycin B
z.B.: ein well in 75 cm²-flask
- „polyclonal“ selection and screening of Hygromycin B-resistants
- Zusätzliche Tests nach der Hygromycin B-selektion:
 - 1) Zeocin-Sensitivity
 - 2) Lack of β-galactosidase activity
 - 3) Expression des gene of interest

Transfection protocol

2 days Transfection

+

Ca.:

7+ Tage