Blasticidin S HCl (#B001)

Protocol:

1. Seed cells of the parental cell line in a 24-well plate at different densities (50,000 – 100,000 and 200,000 cells/ml) and incubate the cells for 24 hours at 37°C.
2. Remove medium and then add medium with varying concentrations of antibiotic (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 µg/ml) and incubate at 37°C.
3. Refresh the selective medium every 3-4 days and observe the percentage of surviving cells over time (e.g. by EMA vs Hoechst staining or MTT assay).
4. Determine the lowest concentration of antibiotic that kills a large majority of the cells within 14 days. This concentration should be used for selection of a stable transfected cell line.
5. If necessary, repeat the experiment to narrow the antibiotic concentration range.

Plasmid DNA Transfection Protocol

Background:

Once the appropriate antibiotic concentration to use for selection of the stable transfected cells has been determined by performing a kill curve, the next step is to generate a stable cell line by transfection of the parental cell line with a plasmid containing the gene of interest and an antibiotic resistance gene.

Plasmid DNA Transfection Protocol:

1. Seed the parental cell line in 24-well plate and incubate for 24h at 37°C.
2. Transfect the parental cell line the next day at 80% confluency with the construct (e.g. using calcium phosphate etc…) and include a sample of untransfected cells as a negative control. Incubate at 37°C in C02.
3. After transfection (6h to 24h depending on the transfection method used), wash the cells once with 1X PBS and add fresh medium containing the selection antibiotic to the cells. Use the appropriate antibiotic concentration as determined from the kill curve.
4. Check, refresh, and expand the cells in selection medium every 2-3 days until you have enough cells for limited dilution (confluency in T25 flask or 10 cm dish).

QC

Seed 24-wells with insert and determine the transfection efficiency by immunostaining:

1. Grow cells on insert in a 24-well plate until well is confluent.
2. Remove medium and wash cells with 1X PBS.
3. Fix cells with methanol or paraformaldehyde and wash with 1X PBS.

4. Add primary antibody in 24-well against protein of interest and incubate at 37°C for 1 hour (depending on antibody).
5. Wash cells with 1X PBS.
6. Add secondary antibody in 24-well and incubate at 37°C for 1 hour depending on antibody).
7. Wash with 1X PBS.
8. Remove insert from 24-well plate and affix to microscopy slide with nail polish or other suitable adhesive.
9. Determine the percentage of transfected cells with fluorescence microscope.

Selection of Stable Transfected Cell Lines Protocol

Background:

Once the cells have been successfully transfected, the next step is to seed and select the transfected cell line in a single 96-well plate to select pure colonies by limited dilution as outlined below:

Protocol:

1. Seed the transfected cells in 96-well plates in 10% conditioned medium
    - 2x96 well plate with 0.1 cell per well
    - 2x96 well plate with 0.5 cell per well
    - 2x96 well plate with 1 cell per well
2. Incubate the cells for 24h.
3. Remove medium and add conditioned selection medium containing selection antibiotic at the pre-determined concentration required for your cell line. Incubate 96-well plates at 37°C with C02.
4. Check the plates every day for colonies. Colony formation depends on proliferation rate of the cell line and can take anywhere from 3 days to 1 week.
5. Refresh selective medium every 3-4 days until colonies appear.
6. Select the wells with only one single colony. Make sure colonies are not growing in clumps as they will be less sensitive to the antibiotic.
7. When a well contains a single colony, transfer the colony to a 24-well in selection medium and so on until you have enough cells for freezing and storage in liquid nitrogen. Use the appropriate antibiotic concentration as determined from the kill curve.

QC

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Seed 24-wells with insert for an immunostaining to determine percentage of cells expressing the gene of interest to be able to identify a 100% pure clone. You can also use Western blotting, flow cytometry or another technique depending on the cell line used.

Seed 24-wells with insert and determine the expression level of the gene of interest by immunostaining:

1. Grow cells on insert in a 24-well plate until well has confluent growth.
2. Remove medium and wash cells with 1X PBS.
3. Fix cell with methanol or paraformaldehyde and wash with 1X PBS.
4. Add primary antibody in 24-well against protein of interest and incubate at 37°C for 1 hour (depending on antibody).
5. Wash cells with 1X PBS.
6. Add secondary antibody in 24-well plate and incubate at 37°C for 1 hour (time depends on antibody type).
7. Wash cells with 1X PBS.
8. Remove insert from 24-well plate and affix to microscopy slide with nail polish or other appropriate adhesive.
9. Determine the percentage of transfected cells with fluorescence microscope.