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Protocol Information

YEX™ Cryopreservation Protocol

Background
YEX can replace fetal bovine serum (FBS) in freezing medium as well as in growth medium. Cells are frozen in a mixture of 90% YEX and 10% dimethyl sulfoxide (DMSO), in the same way as the common 90% FBS + 10% DMSO formulation, and thawed back into complete medium containing YEX.

In head-to-head testing with two hybridoma clones, cells frozen in 90% YEX + 10% DMSO grew as well after thawing as cells frozen in 90% FBS + 10% DMSO. In separate testing, chick embryo fibroblasts frozen in YEX showed approximately 90% recovery.

Please note: the recommendation not to exceed 10% YEX applies to growth medium only. Freezing medium is used briefly and at a much higher concentration, so 90% YEX is correct here.

Contents

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IMPORTANT NOTE: Use the YEX formulation your cells are grown in, **work quickly** once cells are in freezing medium, and always **remove the DMSO** after thawing (Sections 3 and 4).

CATALOGUE NUMBER: Adherent: YEX-003 (500 mL), YEX-006 (50 mL); Suspension: YEX-005 (500 mL), YEX-007 (50 mL)

PRODUCT NAME: YEX for adherent cells; YEX for suspension cells

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SAFETY: Handle using aseptic cell culture technique. DMSO passes readily through skin and can carry other substances with it.
Wear gloves and follow your institution's safety and biosafety requirements.

STORAGE: YEX: store at –80 °C (–20 °C is acceptable). Freezing medium: prepare fresh on the day of use and keep at 2–8 °C.
Frozen cells: store in liquid nitrogen (vapour phase) for long-term storage.

1. MATERIALS:

- YEX, thawed as described in the YEX Receiving, Thawing and Passaging Protocol (use the adherent or suspension formulation your cells are grown in)
- Cell culture grade DMSO
- Complete growth medium containing YEX, pre-warmed to 37 °C (for thawing)
- Sterile cryovials, labelled with cell line, passage number, date and density
- Controlled-rate freezing container (for example, an isopropanol-based or alcohol-free cell freezing container) that cools at approximately –1 °C per minute
- 1× PBS, and trypsin-EDTA for adherent cells

2. PREPARING FREEZING MEDIUM:

1. Prepare freezing medium fresh on the day of use: **90% YEX + 10% DMSO**. For example, for 10 mL, combine 9 mL of YEX with 1 mL of DMSO.
2. Add the DMSO slowly to the YEX while gently swirling or inverting. **Do not vortex.** Mixing DMSO with aqueous solutions releases heat, so adding it slowly prevents local warming.
3. Chill the freezing medium at 2–8 °C or on ice before use.

3. FREEZING CELLS:

1. Use healthy cells in the exponential (log) growth phase with viability above 90%.
2. Harvest the cells:
    1. **Suspension cells:** transfer the culture to a sterile centrifuge tube and spin down at 300–400 ×g for 5 minutes.
    2. **Adherent cells:** detach with trypsin-EDTA, add medium or 1× PBS, and spin down at 350 ×g for 5–7 minutes. Removing the trypsin is optional, as described in the main protocol.

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3. [OPTIONAL} Count the cells. Resuspend a small sample in 1× PBS or medium **without YEX** for counting, as components in YEX can interfere with trypan blue staining.
4. [OPTIONAL] Spin the cells down again and remove the supernatant.
5. Gently resuspend the cell pellet in cold freezing medium at the density you normally use for your cell line (typically 1 to 5 million viable cells per mL; hybridomas are often frozen at 5 to 10 million cells per mL).
6. Dispense 1 mL into each labelled cryovial. **Work quickly:** aim to have the vials in the freezing container within 10 minutes of adding the freezing medium, as DMSO is harmful to cells at room temperature.
7. Place the vials in the controlled-rate freezing container and transfer it to a –80 °C freezer overnight.
8. Within 24–48 hours, transfer the vials to liquid nitrogen (vapour phase) for long-term storage. **Storage at –80 °C is suitable for short periods only.**

4. THAWING CELLS:

1. Pre-warm complete growth medium containing YEX to 37 °C. Add 9 mL to a sterile centrifuge tube for each vial.
2. Remove the vial from liquid nitrogen and thaw it quickly in a 37 °C water bath, swirling gently, until only a small ice crystal remains (about 1–2 minutes).
3. Wipe the vial with 70% ethanol and move it to the biosafety cabinet.
4. Transfer the cells **drop by drop** into the 9 mL of pre-warmed medium while gently swirling the tube. Adding the cells slowly reduces osmotic shock.
5. Spin down at 300 ×g for 5 minutes to **remove the DMSO**, then discard the supernatant.
6. Gently resuspend the cells in fresh complete medium containing YEX and transfer them to a culture vessel at your usual seeding density.
7. The next day, check viability and attachment. For adherent cells, replace the medium after 24 hours to remove dead cells and debris.

5. TIPS FOR BEST RESULTS:

- Cells may take one or two passages after thawing to return to their normal growth rate. This is normal for cryopreserved cells and is not specific to YEX.
- Freeze several vials from the same culture at once, and thaw one vial a week later to confirm recovery before relying on the stock.
- Do not refreeze freezing medium or reuse it for a later freeze.
- Avoid temperature fluctuations during storage. Minimise the time vials spend outside liquid nitrogen when retrieving other vials.
- If your cells were previously frozen in FBS, they can be thawed directly into complete medium containing YEX.

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