www.yexbio.com
M7-122, 2075 Bayview Ave, Toronto, ON, M4N 3M5

## Protocol Information

# YEX™ for Adherent Cells and Suspension Cells

**Background**

YEX is an egg-based alternative to fetal bovine serum (FBS) and is designed to be used the same way as FBS in cell culture. Because it is not a blood product, it does not contain all of the factors found in FBS. For example, FBS contains alpha-1 antitrypsin, which neutralizes the trypsin used to passage adherent cells. Egg yolk has no direct equivalent, so we strongly recommend washing away trypsin at the end of passaging. Adherent cells usually still attach if this step is skipped, but how well they attach depends on how much trypsin is used, so washing gives the most consistent results. Removing trypsin is not required for suspension cells.

Components in YEX can also interfere with viability dyes such as trypan blue. For accurate counts, resuspend cells in PBS or media without YEX before counting. Both steps are described in Section 5, “Cell Passaging and Counting.”

We hope you enjoy using YEX for your cell culture activities and that it meets your needs.

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5. Cell passaging and counting

**IMPORTANT NOTE:** YEX does not contain trypsin inhibitors and may interfere with trypan blue staining. Please follow Section 5 carefully for accurate **cell counting** and **proper passaging** techniques.

**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.
Follow your institution's biosafety requirements for the cells being cultured.

**STORAGE:** YEX is shipped on dry ice.
Store at –80 °C (–20 °C is acceptable) until ready to use.

## 1. RECEIVING YEX:

YEX is shipped on dry ice in a 500 mL bottle or a 50 mL tube. On arrival, check that the package is still frozen, remove YEX from the dry ice and place it in the freezer immediately. For optimal performance, store YEX at –80 °C. If –80 °C is unavailable, –20 °C is acceptable.

## 2. THAWING YEX:

**500 mL bottle:**

1. Place the bottle in a 37 °C water bath. Gently invert every 20 minutes until only a small ice crystal remains, then complete thawing at room temperature (total time: 90–120 minutes).
2. Gently invert the thawed bottle 10 times to mix. **Do not shake or vortex**, as this reduces potency and causes precipitates to form.
3. In a biosafety cabinet, aliquot YEX into sterile 50 mL tubes and immediately return them to –80 °C.
4. Thaw one 50 mL aliquot as described below and proceed to Section 3.

**50 mL tube:**

1. Place the tube in a 37 °C water bath. Gently invert every 5 minutes until only a small ice crystal remains, then complete thawing at room temperature (total time: 15–20 minutes).
2. Gently invert the thawed tube 10 times to mix. **Do not shake or vortex.**
3. Proceed to Section 3.

**NOTE:**

- Do not keep undiluted YEX at room temperature or in the refrigerator longer than necessary, as this can cause degradation and flocculence.
- Avoid repeated freeze–thaw cycles, which reduce potency. A single aliquoting step, as described above, is fine.

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## 3. MAKING COMPLETE MEDIA:

1. Pre-warm the base medium (e.g. DMEM or RPMI) in a 37 °C water bath.
2. Add YEX to the base medium at the desired concentration. **We recommend 0.5–10% (v/v); do not exceed 10%.** For example, 50 mL of YEX in 450 mL of medium gives 10%. Cells currently grown in 10% FBS can often grow in less YEX, so we suggest also testing 5%.
3. Add other supplements (e.g. antibiotics or growth factors) according to your usual protocol.
4. Gently invert the bottle 10 times to mix. **Do not vortex.**

**NOTE:**

- Complete media containing YEX may look slightly cloudy. This is not contamination: it comes from the natural fats and lipoproteins that support cell growth. Under the microscope, the field of view is clear and cells are easy to see.
- Small floating “flake-like” particles may appear during culture, caused by natural fats aggregating with temperature changes. They do not harm cell viability.

## 4. STORING COMPLETE MEDIA:

1. Store complete media in the refrigerator at 4–8 °C for up to one month. Do not allow it to cool below 4 °C.
2. Avoid repeated freeze–thaw or warming and cooling cycles. If you use small volumes often, aliquot the complete media and warm only what you need.

## 5. CELL PASSAGING AND COUNTING METHOD FOR CELLS GROWING IN MEDIA CONTAINING YEX:

**Suspension cells:**

1. Monitor the culture to ensure cells are at the appropriate density for passaging.
2. Transfer the desired volume of cell culture to a sterile centrifuge tube.
3. Spin down the cells at 300-400xg for 5 minutes.
    - Optional: Cells may also be directly passaged in fresh media without centrifugation.
4. Carefully aspirate the supernatant without disturbing the cell pellet.

**For Passaging:**

5. Resuspend the cell pellet in a pre-determined volume of fresh, complete media containing YEX.
6. Transfer the resuspended cells back into a new culture vessel at the desired density.
7. Place the culture vessel back in the incubator and monitor cell growth.

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**For Counting:**

5. Resuspend the cells in 1× PBS or media **without YEX**.
6. Count according to your lab protocol.

**Adherent cells:**

1. Remove old media and wash cells with PBS.
2. Add trypsin-EDTA solution to cover the cell monolayer.
3. Incubate at 37°C until cells detach.
4. Add complete media and resuspend cells.
    - 1× PBS can also be used at this step.
5. Transfer cell suspension to a centrifuge tube.
6. Spin down at 350xg for 5-7 minutes and remove supernatant
    - **Strongly recommended: this removes trypsin, which YEX does not neutralize. Cells usually still attach without this step, but attachment depends on how much trypsin is used.**

**For Passaging**

7. Resuspend cell pellet in fresh complete media containing YEX.
8. Plate cells at desired density in a new culture vessel with complete media.

**For Counting:**

7. Resuspend cell pellet in 1× PBS or media **without YEX** and continue according to your lab counting protocol.