YEX™ for Chick Embryo Fibroblasts

Alternative to FBS for Trypsin Inactivation and Cryopreservation

Summary

The daily production of chicken embryonic fibroblasts (CEFs) requires continuous cell expansion, enzymatic dissociation, cryopreservation, and post-thaw recovery. Fetal bovine serum (FBS) or fetal calf serum (FCS) is routinely used for CEF culture, cryoprotection, and inhibition of trypsin activity following tissue digestion. Given interest in serum alternatives, the potential use of YEX for trypsin inactivation and cryopreservation was evaluated. YEX™ is an egg-based alternative for FBS manufactured using SPF egg yolks. Internal studies demonstrated that supplementation with 10% YEX was as effective as 10% FBS in inactivating trypsin following enzymatic digestion of chick embryo tissues. Maximum cell recovery post-trypsin inactivation was observed when using 50% YEX. Internal studies on cryopreservation demonstrated that YEX achieved maximum 75-80% total cell recovery in primary CEFs post-thawing. YEX was also superior to FBS at the lowest concentrations tested in these cryopreservation post-thawing studies.

What This Means

YEX is a viable drop-in alternative to FBS for trypsin inactivation during the manufacturing process of CEFs. Furthermore, YEX is a viable drop-in alternative for FBS for cryopreservation and cold shipping of CEF products.

Use Case #1: Head-to-head comparison of YEX versus FBS for trypsin inactivation

Day 10 chick embryos were used as the source of CEFs. Tissues were extracted and CEFs isolated as per the protocol provided in Appendix I. Tissues were digested with Trypsin (0.25%, Wisent Inc.) and then inactivated with various concentrations of either FBS (Wisent Inc.) or YEX (YEX Inc.). After 15mins of incubation live and dead cells were counted.

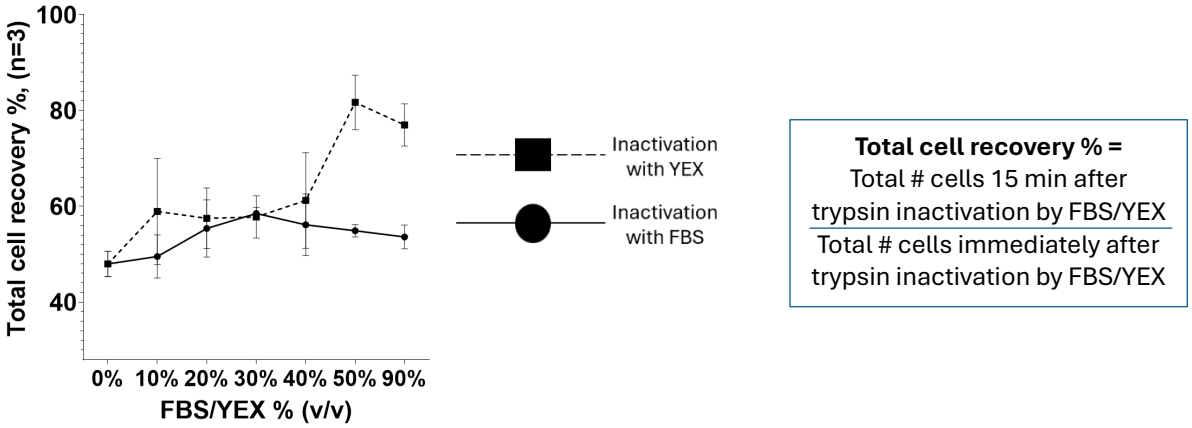
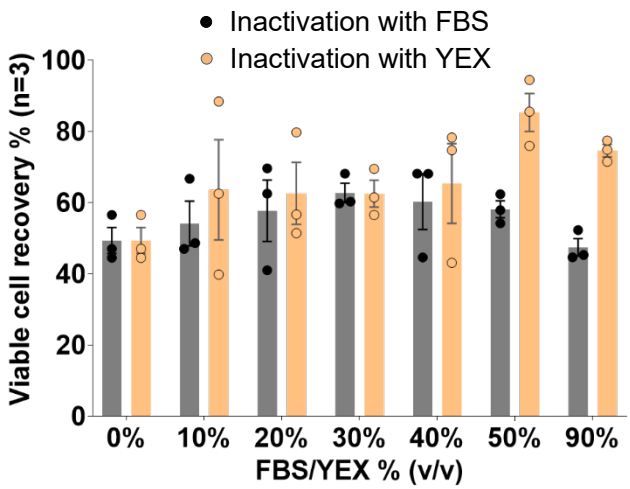


Figure 1. Proportion of total CEFs after trypsin inactivation by FBS or YEX. Various percentages of FBS or YEX were used in DMEM. The total number of cells was determined using a Vi-Cell XR Cell Counter (Beckman Coulter Inc.). The percentage of recovered total CEFs was calculated using the number of total cells counted immediately after trypsin digestion divided by trypsin inactivation for 15 mins @ 37°C using either FBS or YEX.

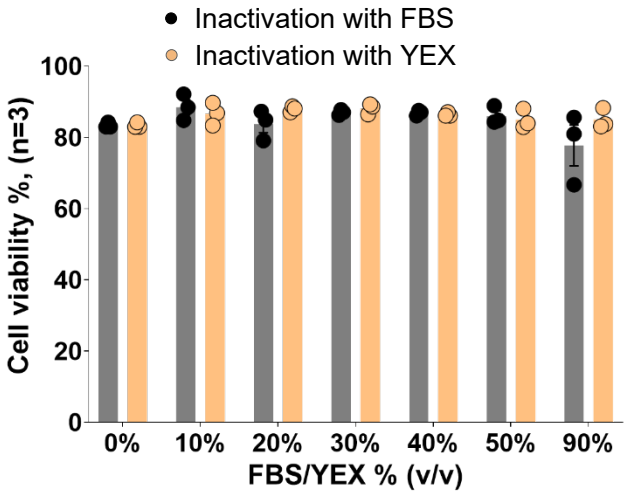
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Viable cell recovery % =
viable cells 15 min after trypsin inactivation by FBS/YEX
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viable cells immediately after trypsin inactivation by FBS/YEX

Figure 2. Proportion of viable CEFs after trypsin inactivation by FBS or YEX. Various percentages of FBS or YEX were used in DMEM. The number of viable cells was determined using a Vi-Cell XR Cell Counter (Beckman Coulter Inc.). The percentage of recovered viable CEFs was calculated using the number of viable cells counted immediately after trypsin digestion divided by trypsin inactivation for 15 mins @ 37°C using either FBS or YEX.



Cell viability % =
viable cells 15 min after trypsin inactivation by FBS/YEX

Total # cells after 15 min of trypsin inactivation by FBS/YEX

Figure 3. Overall viability of recovered CEFs after trypsin inactivation by FBS or YEX. Various percentages of FBS or YEX were used in DMEM. Both total and viable number of cells were determined using a Vi-Cell XR Cell Counter and the Trypan Blue viability dye (Beckman Coulter Inc.). The percentage of CEF viability was calculated using the recovered number of viable and total cells counted immediately after trypsin inactivation by either FBS or YEX

Use Case #2: Head-to-head comparison of YEX versus FBS for cryopreservation

Day 10 chick embryos were used as the source of CEFs. Tissues were extracted and CEFs isolated as per the protocol provided in Appendix I. Tissues were digested with Trypsin (0.25%) and then inactivated with 10% of either FBS (Wisent Inc.) or YEX (YEX Inc.) and then cryopreserved with various concentrations of either FBS/YEX. After 2 days in liquid N_2, live and dead cells were counted. Then cells were plated in either FBS or YEX respectively at 37°C with 5% humidity. Cell morphology was observed after Day 3.

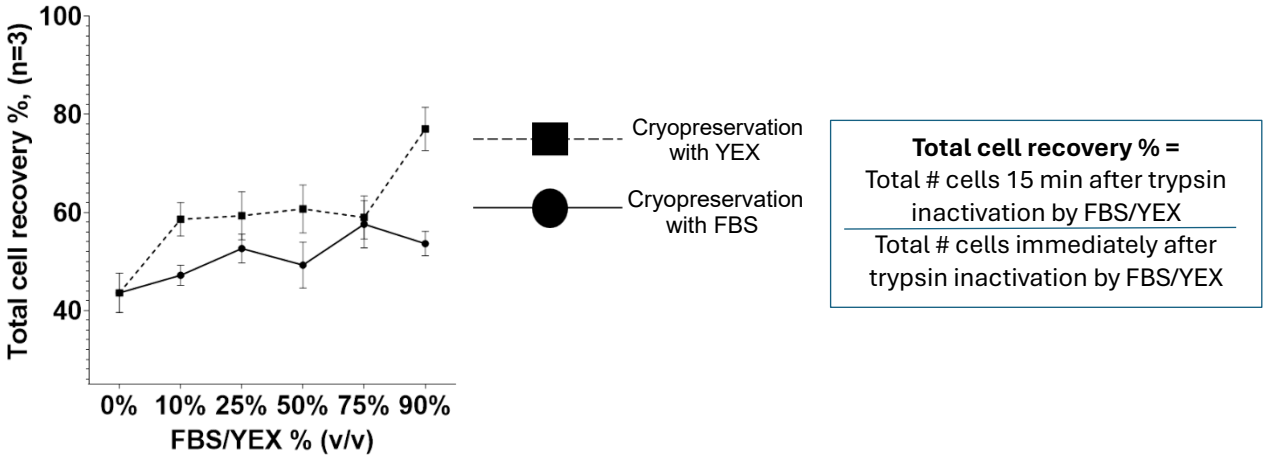


Figure 4. Proportion of total CEFs after cryopreservation in FBS or YEX. Various percentages of FBS or YEX used in 10% DMSO cryopreservant and DMEM vehicle. The total number of cells was determined using a Vi-Cell XR Cell Counter (Beckman Coulter Inc.). The percentage of recovered total CEFs was calculated using the number of total cells counted immediately after trypsin digestion divided by recovered total cells after cryopreservation with either FBS or YEX.

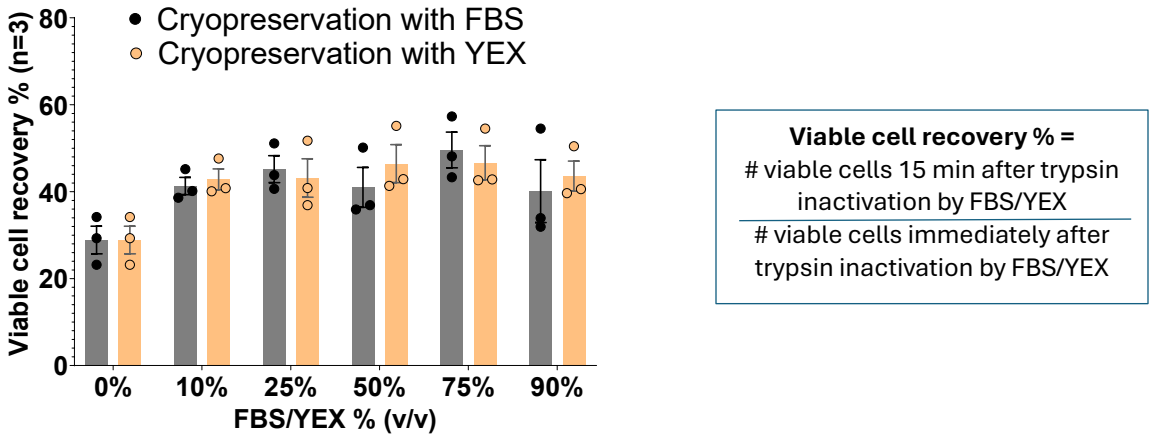
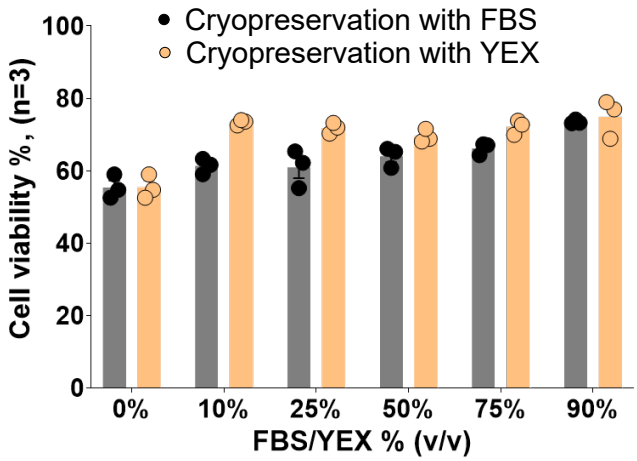


Figure 5. Proportion of viable CEFs after cryopreservation in FBS or YEX. Various percentages of FBS or YEX used in 10% DMSO cryopreservant and DMEM vehicle. The number of viable cells was determined using a Vi-Cell XR Cell Counter (Beckman Coulter Inc.). The percentage of recovered viable CEFs was calculated using the number of viable cells counted immediately after trypsin digestion followed by recovered viable cells after cryopreservation with either FBS or YEX.



Viable cell recovery % =
viable cells 15 min after trypsin inactivation by FBS/YEX
/
viable cells immediately after trypsin inactivation by FBS/YEX

Figure 6. Overall viability of recovered CEFs after cryopreservation in FBS or YEX. Various percentages of FBS or YEX were used in 10% DMSO cryopreservant and DMEM vehicle. Both total and viable number of cells were determined using a Vi-Cell XR Cell Counter and the Trypan Blue viability dye (Beckman Coulter Inc.). The percentage of CEF viability was calculated using the number of recovered viable and total cells counted after cryopreservation in either FBS or YEX.

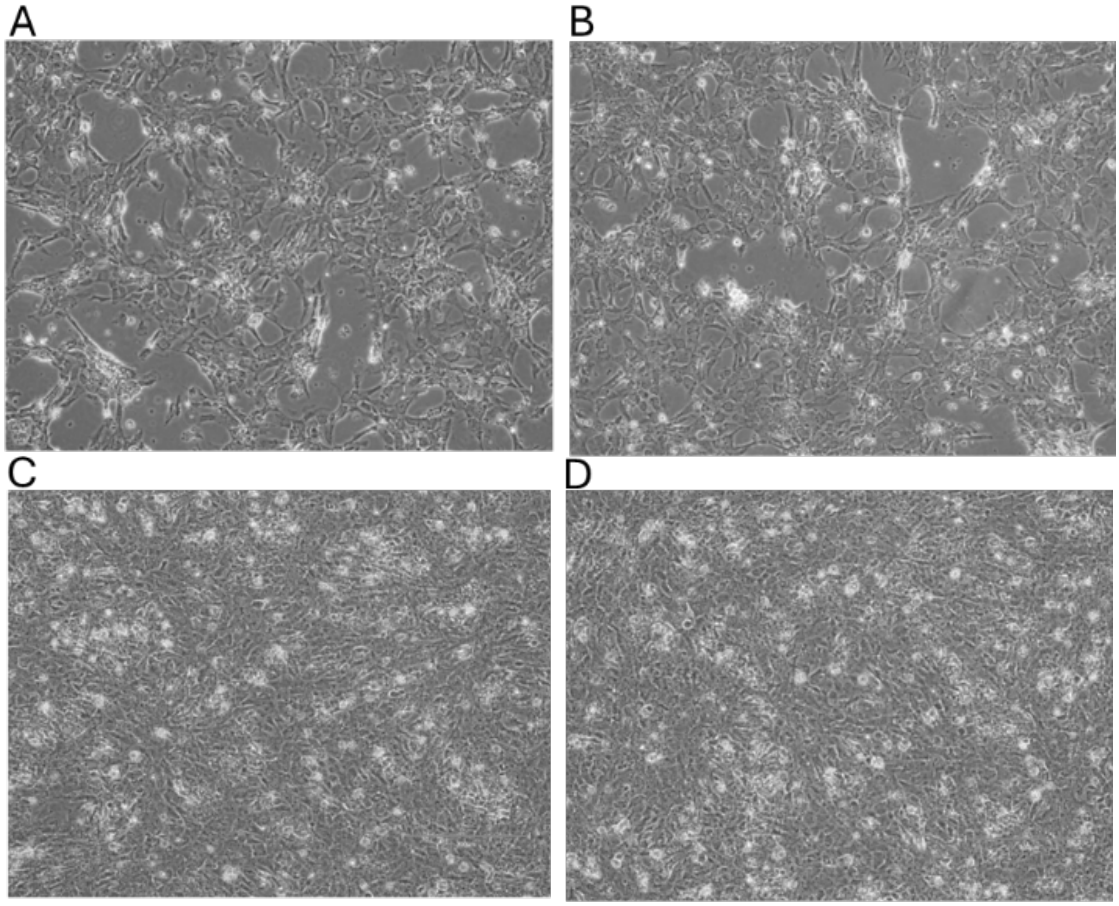


Figure 7. Visual morphology of cultured cells at t=3 days post-cryopreservation in FBS or YEX. Various percentages of FBS or YEX were used in 10% DMSO (DMEM). A) Cryopreserved in 0% FBS and regrown in 10% FBS. (B) Cryopreserved in 0% YEX and regrown in 10% YEX. (C) Cryopreserved in 10% FBS and regrown in 10% FBS. (D) Cryopreserved in 10% YEX and regrown in 10% YEX. The observed cell growth of CEFs cryopreserved at 25%, 50%, 75%, and 90% in 10% FBS or YEX was indistinguishable from the corresponding images of CEFs cryopreserved at 10%.

Technical Inquires

More information is available regarding CEF growth in vitro when comparing YEX to FBS. This information may be applicable from seed train phases to final virus production phases. If interested, please contact info@yexbio.com.

Appendix I

Protocol for CEF Production

Biological material and chemicals

- Fertilized chicken SPF eggs, 9-11 days old
- Fetal bovine serum (FBS)
- L-glutamine 200 mM
- Penicillin/Streptomycin
- MEM Non-Essential Amino Acid Solution 100× (NEAA)
- YEX media

Substrates and solutions

- DMEM substrate
- DMEM medium with FBS (500 ml of FBS, 5 ml of L-glutamine, 5 ml of Penicillin/Streptomycin and 5 ml of NEAA are added to 500 ml of the medium)
- Trypsin-EDTA (0.5 g/L trypsin and 0.2 g/L EDTA)
- Tripan blue 0.4%

Laboratory material

- Automatic single-channel pipette (10-100 µl volume) and tips
- Automatic single-channel pipette (100-1000 µl volume) and tips
- Pipettor
- Disposable pipettes (10 and 25 ml)
- Centrifuge tubes (15 ml)
- Sterilized Petri dish
- Sterilized tweezers (2 pieces)
- Sterilized scissors (1 piece)
- Sterilized 150 ml glass cup
- Sterilized glass cup of 300 ml or 500 ml (2 pieces)
- 10ml Sterilized Disposable Plastic Syringe
- Sterilized magnet
- Sterilized triple gauze
- Cell culture bottles or microtitration plates

Equipment

- Cell Counting Chamber (Beckman Coulter Inc.)
- Magnetic stirrer
- Centrifuge
- Inverted microscope
- Egg incubator (37,5 °C and 50 % humidity)
- Ovoscope
- Cabinet for sterile operation with vertical air flow

Preparation of primary and secondary culture of chicken fibroblasts

The procedure for the preparation of a primary culture of PF from 3 embryos is described. Adjust the number of embryos according to the number of cells needed for further testing.

1. Incubate eggs for 9-11 days in an incubator at 37.5 °C and 50% humidity.
2. Inspect the eggs with an ovoscope and separate 3 eggs from which you will prepare the PF culture.
3. Disinfect the eggshell with 70% alcohol.
4. Pour 50 ml of PBS, preheated to 37 °C, into a 150 ml glass.
5. Use scissors to gently tap through the eggshell in the area of the air bladder and cut the shell in a circular way above the air bladder. Try to cut the shell in one piece, so that as little eggshell as possible crumbles on the white membrane underneath. Use tweezers to remove the white membrane.
6. Pour DMEM medium into a Petri dish to cover the bottom and use the second tweezers to remove the embryo into the pelvis.
7. Remove the embryo's head, limbs and entrails and transfer the carcass to a cup with trypsin until you have processed all the embryos.
8. Squeeze all the carcasses together through a 10 ml syringe (without a needle) into the same glass with trypsin and insert the magnet into the glass.
9. Stir on a magnetic stirrer for 15 minutes at 37 °C, then profile through triple sterilized gauze into a glass glass.
10. Pour the suspension from the beaker into a centrifuge and centrifuge for 10 minutes at 95 × *g* (1000 rpm on a Hettich EBA 20 centrifuge).
11. Obtain the first cell count (just after trypsinization)
12. Carefully cast the supernatant and resuspend the precipitate with 5-10 ml of DMEM with either FBS or YEX separately.
13. Profile the suspension through a triple sterilized gauze into a new glass cup, then pour it into a centrifuge tube.
14. If necessary, count the cells or distribute the cell suspension without counting them into cell culture bottles or microtitration plates. Dilute the cell suspension with DMEM with FBS or YEX.
15. Incubate the cell bottles/plates at 37 °C and 5% CO_2. After 24 hours, inspect the cells. If the cells do not need to be subcultured, after 24 hours change the medium or use it for whatever is needed. If cells need to be subcultured, prepare a secondary culture of PF as described below.
16. If you are using test cells in microtiter plates, subculture the primary PF culture by pouring the substrate from the cell culture bottle. The cells are then first rinsed with trypsin-EDTA (e.g. 5 ml for a 162 cm_2 bottle) preheated to 37 °C. Then add another 5 ml of trypsin-EDTA and wait for the cell layer to peel off. If it does not peel off, incubate for a few minutes at 37 °C. Resuspend the cells with a pipette and use it for whatever it takes.