Expi293F cells

ThermoFisher Scientific, A14527

https://www.thermofisher.com/order/catalog/product/A14527?SID=srch-srp-A14527#/A14527?SID=srch-srp-A14527

General Information

Highly transfectable variant of 293F cell line maintained in suspension culture and capable of growing to high density in Expi293 Expression Medium (ThermoFisher Scientific, A1435102). Media requires no supplements, but is extremely light sensitive- use and store media protected from light. Make aliquots of media to avoid repeated warming/cooling.

Grow cells in orbital shaker at 37°C, 120-125 rpm with 8% CO_2. Reduce speed to 90 rpm if growing culture volumes above 400 ml.

Doubling time is 24-25 hours. The first subculture should be when cell density reaches 1-3 x 10^6 viable cells/ml. For general maintenance, subculture when cells reach 3-5 x 10^6 cells/ml. Seed shaker flasks at 0.4-0.5 x 10^6 cells/ml in pre-warmed media every 3 days, or 0.3-0.4 x 10^6 cells/ml every 4 days. Do not grow above 5 x 10^6 cells/ml, and do not subculture cells before reaching 3 x 10^6 cells/ml. Generally use 125 or 250 ml flasks and maintain a 1:3 or 1:4 culture volume to flask ratio (i.e. 30-40 ml cell suspension in 125 ml flask). Maintain cells in culture for no more than 30 passages.

Freeze cells in fresh Expi293 Expression Medium with 10% DMSO at a density of 1 x 10^7 viable cells/ml.

Rinse used flasks with 10% acetic acid followed by 10 flask volumes of dH20. After drying, cover with foil and autoclave for re-use. For vented caps, rinse with 70% EtOH (avoiding center) and leave in hood under UV light overnight to sterilize. Wrap in foil for storage.

Transfection of Expi293F cells with Spike v1 and PEI
Protocol from Fang XT et al., Biological Procedures Online 2017
(written for 500 ml transfection volume; scale as appropriate)

1. Subculture at least 3 passages after thawing cells before use in transfection experiments.

2. Approximately 24 hours before transfection, passage cells at 2.0-2.5 x 10^6 cells/ml. Place flask(s) on an orbital shaking platform at 125 rpm and 37°C, 8% CO_2.

3. On the day of transfection, cell density should be ~4-5 x 10^6 cells/ml. Count cells and pellet 500 x 10^6 viable cells. Resuspend in 25 ml fresh Expi293 Expression Medium containing 0.1% Pluronic F-68 (Fisher Scientific, ICN2750049) to reduce shear forces during transfection. Transfer cells to a 125 ml flask.
    a. Cells resuspended in transfection medium at a final concentration of 20 x 10^6 viable cells/ml.
    b. Viability must be >95%.

4. Add 625 ug DNA to the culture, dropwise while swirling.

5. After mixing DNA with cells, add 1.875 ml PEI Max (40 kDa at 1 mg/ml) dropwise while swirling.

6. Incubate cells on orbital shaking platform for 3 hours.

7. Dilute cells to 1 x 10^6 cells/ml by adding 475 ml pre-warmed Expi293 Expression Medium.

8. Add 0.5 M VPA (Sigma, P4543) to a final concentration of 3.5 mM (VPA is an HDAC inhibitor that slows cell growth while maintaining cell viability, and can improve protein production).

9. Incubate 3-4 days at 125 rpm and 37°C, 8% CO_2 without handling cells.

Optimization

1. Ratio of DNA to PEI in above protocol is 1:3, and more protein was produced via this protocol than the traditional PEI transfection protocol outlined for FreeStyle 293-F cells.
    a. May want to test additional ratios using this protocol and/or the traditional PEI transfection protocol for each construct.
    b. Suggest ratios of 1:2, 1:3, 1:4, and 1:9 with use of 0.8-1.25 ug DNA per ml of transfection volume for the traditional protocol.
2. Maximal protein yield could be between 1-7 days post-transfection. Initial test should be much smaller scale (30 ml) and take daily samples to determine peak of protein production and monitor cell viability.
    a. **Maximal yield may not correlate with protein quality/homogeneity.
3. VPA could range between 0.5 and 3.5 mM, with addition at 8-24 hours post-transfection (see You M et al., *Biosci Biotechnol Biochem* 2013).
4. Pluronic F-68 (Fisher Scientific, ICN2750049) can be added to reduce shear forces during transfection (and subculturing, if foam is generated). Add to a final concentration of 0.1% when resuspending cells for transfection.
5. Thermo recommends transfection with their proprietary reagent- ExpiFectamine. Transfection with ExpiFectamine followed by addition of Enhancers 1 and 2 did produce a 4-5 fold higher yield than that observed by PEI transfection.

a. Cost per liter is around $700 for Expifectamine and $4 for PEI.
b. Cost of media may offset the cost of the transfection reagent, if need to transfect 5 L with PEI to achieve same yield as 1 L with ExpiFectamine.
c. Additional consideration is whether the higher yield provides protein of sufficient quality. May be a more heterogeneous population, with problems of protein folding or PTMs if the cell is overwhelmed by the amount of protein being produced.