

Culture Medium Preparation for Cell lines T7 and C22 (Clara)

The fully supplemented culture medium for cell lines T7 (ECACC cat no. 07021402) and C22 (Clara) (ECACC cat no. 07021401) consists of:

DMEM + 2mM Glutamine + 4% Foetal Bovine Serum (FBS) +

0.25 µg/ml	Endothelin-1
0.01 µg/ml	Interferon gamma
10 µg/ml	Insulin
5 µg/ml	Transferrin
7.5 µg/ml	Endothelial Cell Growth Supplement (ECGS)
0.025 µg/ml	Epidermal Growth Factor
0.36 µg/ml	Hydrocortisone
0.02 µg/ml	T3

Stock Solutions of Supplements

The information below regarding stock solutions is provided to aid with preparation of the fully supplemented culture medium. Please note that this is a guide only – it is necessary to refer to the supplier's information regarding the reconstitution and storage of the supplements.

The supplements should be prepared using sterile precautions as they cannot be filtered – some factors stick to the filter and are lost.

- **Endothelin-1 human, porcine Sigma cat no. E7764**

Make up the stock concentration to 0.2 mg/ml in 1% acetic acid.

To 100 ml of culture medium add 125 µl of the stock solution to make the final concentration in the culture medium 0.25 µg/ml Endothelin-1.

- **Interferon gamma Sigma cat no. I3265**

Make up the stock concentration to 10µg/ml in phosphate buffered saline (PBS).

To 100 ml of culture medium add 100 µl of the stock solution to make the final concentration in the culture medium 0.01 µg/ml interferon gamma.

- **Insulin Sigma cat no. I1882**

Make up the stock concentration to 10 mg/ml of 1% acetic acid.

To 100 ml of culture medium add 100 µl of the stock solution to make the final concentration in the culture medium 10 µg/ml insulin.

- **Transferrin BD Biosciences cat no. 354204**

Make up the stock concentration to 10µg/µl in sterile water.

To 100 ml of culture medium add 50 µl of the stock solution to make the final concentration in the culture medium 5 µg/ml transferrin.

- **Endothelial Cell Growth Supplement (ECGS) BD Biosciences cat no. 356006**

Make up the stock concentration to 15 µg/µl in sterile serum free medium.

To 100 ml of culture medium add 50 μ l of the stock solution to make the final concentration in the culture medium 7.5 μ g/ml ECGS.

- **Epidermal Growth Factor** **BD Biosciences cat no. 354001**

Make up the stock concentration to 0.1 μ g/ μ l in sterile water.

To 100 ml of culture medium add 25 μ l of the stock solution to make the final concentration in the culture medium 0.025 μ g/ml epidermal growth factor.

- **Hydrocortisone** **BD Biosciences cat no. 354203**

Make up the stock solution to 5 μ g/ μ l

To 100 ml of culture medium add 7.2 μ l of the stock solution to make the final concentration in the culture medium 0.36 μ g/ml hydrocortisone.

- **T3** **Sigma cat no. T2877**

Make up the stock solution to 0.935 mg/ml.

To 100 ml of culture medium add 2.14 μ l of the stock solution to make the final concentration in the culture medium 0.02 μ g/ml T3.