

# TCM<sup>TM</sup> and TM-235<sup>TM</sup> Defined Serum Replacement

## Product Instructions

TCM and TM-235 are fortified, multipurpose serum replacements for the long-term culturing of anchorage dependent and suspension cultures. These serum replacements are effective in research situations such as studying the effects of growth factors or in the manufacturing of cell derived products.

TCM and TM-235 are packaged as a 50X concentrate. Therefore, 10ml will produce 500ml of growth medium, 100ml will produce 5 liters of medium, and 500ml will produce 25 liters of medium.

TCM and TM-235 are stable for a minimum of 6 months after receipt when stored at 2 to 8°C in the 50X concentrate and 30 days when diluted with basal media.

The following recommended protocol is a series of serum dilutions while increasing the concentration of a CELOX serum replacement. Since each cell line has unique characteristics, slight adjustments in cell density, length of adaptation period, etc. may be necessary. Please contact our technical service department for additional information.

### I. Preparation

1. Aseptically add 10ml of the CELOX serum replacement to 500ml of basal medium.
2. Recommended final working concentration is 2%.
3. Add 2-4 mM sterile L-glutamine.
4. Add antibiotics if desired.
5. Add 10 to 20mM sterile HEPES.
6. Add 1mM sodium pyruvate if necessary.
7. The final pH should be 7.2±0.1.

### II. Adaptation of Cells

1. Dilute serum containing medium two-fold with CELOX serum replacement medium.

Example: Adding 5ml of 10% FBS-containing medium with 5ml CELOX serum replacement medium would result in a final serum concentration of 5%.

2. Culture cells for two passages.
3. Dilute serum two-fold again with CELOX medium.

Example: Adding 2.5 ml of 10% FBS with 7.5ml of CELOX medium.

4. Culture cells for at least two passages.
5. Continue to adapt cells in similar manner, each time reducing the FBS concentration by one-half until the serum has been completely eliminated.

**Note:** Some cell lines will not adapt to a level of serum below 0.5% depending on culture conditions. Please contact our technical service department for additional information.

## **Special Notes**

- 1. Anchorage dependent cultures should be monitored for trypsin toxicity when serum levels are less than 1%. Care must be taken not to over-trypsinize some adherent cells.**
- 2. Cells should be maintained in logarithmic growth for optimal results.**
- 3. The pH of the medium should be monitored carefully.**
- 4. Cells growing in a low protein, non-serum media are generally more sensitive to antibiotics, enzymes, hormones, and cytokines.**

**Thus, these components should be adjusted accordingly. In general, a two-fold reduction is appropriate.**

**Any question concerning CELOX products can be addressed directly to our technical service department.**

**Protide Pharmaceuticals**

**Celox Laboratories**

**505 Oakwood Road**

**Suite 200**

**Lake Zurich IL 60047**

**847-726-3100**