

## Product Information

### SERUM REPLACEMENT 3 (50X)

Product Number **S 2640**

Storage Temperature 2 – 8 °C

#### Product Description

Sigma's Serum Replacement 3 (Catalog No. S 2640) is a fortified, multipurpose serum replacement for the long term culturing of anchorage dependant and suspension cultures. This serum replacement is effective in research situations such as: studying the effects of growth factors or in the manufacturing of cell derived products.

Serum Replacement 3 is packaged as a 50X concentrate. Therefore 2 x 10 ml will supplement 1 liter of basal medium and 2 x 100 ml will supplement 10 liters of basal medium.

#### Intended Use

**For Laboratory Use Only. Not for drug household or other uses.**

#### Product Use

The following protocol is recommended for the adaptation of cultures from serum-containing medium to medium containing Serum Replacement 3. This can be accomplished by gradually diluting the serum concentration while increasing the concentration of Serum Replacement 3.

##### 1. Serum-Free Medium Preparation

- Aseptically add 10 ml of Serum Replacement 3 to 500 ml of basal medium. (The final concentration is 2%).
- If necessary, add 2-4 mM sterile L-glutamine (Sigma Catalog No. G 7513).
- Add antibiotics, if desired.
- Add 10 – 20 mM sterile HEPES (Sigma Catalog No. H 0887).
- Add 1 mM sterile sodium pyruvate (Sigma Catalog No. S 8636) if necessary.
- The final pH of the medium should be 7.1 – 7.3.

##### 2. Adaptation of Cells

- Dilute serum containing medium two-fold with serum free medium.  
For example: 5 ml of medium with 10% FBS + 5 ml of serum-free medium would result in a final serum concentration of 5%.
- Culture cells for 2 passages.
- Dilute serum containing medium two-fold again with the serum-free medium.  
For example: 2.5 ml of medium with 10% FBS + 7.5 ml of serum-free medium would result in a final serum concentration of 2.5%.
- Culture cells for 2 passages.  
Continue to passage cells in a similar manner each time reducing the serum concentration by one-half until the serum has been completely eliminated.

#### Special Notes:

1. Some cell lines will not adapt to a serum level below 0.5% depending on culture conditions.
2. Anchorage dependent cultures should be monitored for trypsin toxicity when serum levels are less than 1%.
3. In general, cultures should not exceed a cell density of  $1 \times 10^6$ /ml and cells should be maintained in logarithmic growth.
4. The pH of the medium should be monitored carefully.
5. Cells grown in a low protein, non-serum medium are generally more sensitive to antibiotics, enzymes, hormones and cytokines. These components should be adjusted accordingly - in general a two-fold reduction is appropriate.

#### Product Storage

Serum Replacement 3 should be stored at 2-8 °C. Product label bears expiration date. When diluted in basal medium it is stable for 40 days.

## References

1. Gleave, M., Hsieh, J.T., Gao, C., von Escshnbach, A.C., and Chung, L.W.K. *Cancer Research*, **51**:3753 (1991)
2. Tiffany, C.W., Hoefler, S., Moser, H.W., and Burch, R.M.. *Biochimica et Biophysica Acta*, **1096**:41(1991)
3. Hsieh, J.T., Wu, H.C., Gleave, M., von Escshnbach, A.C., and Chung, L.W.K. *Cancer Research*, **53**:2852 (1993)

KMR 08/01

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.