

## HeLa SPINNER CULTURES

Use HeLa S3 cells which have been adapted for growth in spinner culture.

### **Procedure:**

1. Frozen cells should be thawed into a 150 mm dish containing DME/5% calf serum and allowed to attach and fill out this dish
2. Trypsinize cells, resuspend in S-MEM (Joklik's)/5 % calf serum, and count.
3. Add medium so that cells are at a density of  $2 \times 10^5$  cells/ml and transfer to 500 ml spinner flask. Spin at medium setting, sufficient to maintain good aeration but slow enough to avoid damaging the cells. Do not be concerned if medium initially looks purple; it will turn proper red-orange color as cells grow. Fresh medium should be added daily to maintain cells at  $3-6 \times 10^5$  cells/ml for optimum growth.

### **Comments:**

Spinner flasks should not be filled more than half-way to maintain sufficient aeration; as cell number increases, larger sized flasks will have to be used. Side arm caps must be kept loose to allow for air exchange. Check carefully for contamination - it is often hard to distinguish this from cell debris which accumulates as cells are maintained in spinner culture.

After use, rinse spinner flasks thoroughly with tap water followed by distilled water. Add a small amount of dH<sub>2</sub>O to flasks before autoclaving to avoid breakage during autoclaving - decant prior to use.