INTRODUCTION. FACSmax™ is a gentle and highly effective cell dissociation solution. This proprietary formulation of proteolytic, collagenolytic and DNase enzymes is highly effective in creating single cell suspensions from clumped cell cultures for accurate cell counting, flow cytometry, viral transfection assays, cell sorting, and bioreactor scale-up. In summary, the FACSmax Solution offers the following benefits:

- Dissociates clumped cells in minutes.
- Results in homogeneous single cell suspension.
- Gentle cell disaggregation for maximum cell viability.
- Yields accurate, reproducible cell counts.
- Saves time. No need for extra PBS washing steps.
- Available ready to use.

MATERIALS AND METHODS

A. General Notes

a) Thaw FACSmax™ at 4°C overnight or in a cool water bath. Do not warm to 37°C to avoid inactivation of FACSmax enzymes. 

**NOTE**: After thawing, FACSmax is stable for 2 months at 4°C. **DO NOT** store at room temperature.

b) FACSmax is formulated in standard Dulbecco’s PBS (0.2 g/L KCl, 0.2 g/L KH2PO4, 8 g/L NaCl and 1.15 g/L Na2HPO4) containing 0.5mM EDTA.

c) Cold, 4°C FACSmax can be used directly on cells.

d) For FACS, you may collect the sorted cells into 20% complete medium and passage as usual.

e) This FACS protocol applies to both live and fixed cells.

f) Complete fixing, and all other procedures prior to treatment with FACSmax.

B. Cell Counting

1. Harvest 0.1 ml to 1.0 ml of clumped cell culture as a sample.

2. Add an equal volume of cold FACSmax solution to the cells, and incubate at room temperature for 5-10 minutes.

3. If cells are still clumpy, vortex vigorously for 10-30 seconds.

4. Determine cell count and viability using the trypan blue dye exclusion method or other preferred method.

**NOTE**: Cells treated with FACSmax can be returned to culture and passaged. No additional washes, enzyme inhibitors or changes of media are required.
C. FACS: Suspension Cells

5. Count cells according to Section B, and then determine total number of cells required (we typically use $5 \times 10^6$ cells in 1 ml FACSmax per FACS sample). Transfer the proper volume to a centrifuge tube and pellet cells at 2000 x g for 1-5 minutes.

6. Remove supernatant and resuspend cells in 1 ml cold FACSmax solution. Transfer to 12 x 75 mm polystyrene round bottom snap cap tube and immediately place on ice until the FACS procedure.

D. FACS: Adherent Cells

7. Remove culture medium.

8. Add 1 ml of cold FACSmax solution directly to cells. Use a cell scraper or the large end of a P-200 or P-100 pipette tip to scrape the cells off the bottom of the well. Pipette to mix.

9. Transfer 0.05-0.10 ml to a separate tube containing an equal volume of complete medium (2X dilution) to determine cell count and viability using the trypan blue dye exclusion method or other preferred method.

**NOTE:** Depending on the precision requirements of your experiment, you may either bypass cell counting or further dilute your cells in FACSmax solution to the desired cell concentration for FACS. Typically, we prepare our samples in 6-12 well plates, then harvest and assay the entire cell population in 1 ml of FACSmax solution.

10. Transfer cells from step D8 directly into a 12 x 75 mm polystyrene round bottom snap cap tube and keep on ice until FACS procedure.