# Colony Forming Unit Protocol

Celprogen's Colony Forming Media and Extracellular matrix (ECM) tissue culture system enables one to generate colony forming units for stem cells and cancer stem cells. The colony forming units in stem cells or cancer stem cells is achieved with serum free colony forming culture system. Initially, the cells are grown in complete growth media and are slowly weaned off the serum over the course of one week.

**Procedure:**

1. The cells are grown in complete growth medium with serum in pre-coated tissue culture flasks (T25 or T75) with ECM (animal free conditions). For T25 flasks, use 5 ml of Complete Growth Media, and for T75 flask use 10 ml Complete Growth Media, in flasks pre-coated with ECM. Culture cells at $37^0$C humidified incubator with 5% carbon dioxide. Once the cells have become 60-70% confluent in culture, which usually takes 48 hours, trypsinize the cells.
2. Trypsinization of cells:
    a. For T25 flask with attached cells:
        i. Wash cells with 2mls of 1X PBS solution (Cat# P1408-013), remove 100% 1X PBS solution.
        ii. Add 3 ml of 1X Trypsin EDTA (Cat# T1509-014), and incubate at room temperature for 2- 3 minutes.
        iii. At the end of trypsin incubation, add equal volume (3 ml) of media with serum to neutralize the trypsin.
        iv. Transfer the cell suspension into sterile 15 ml conical tube in tissue culture hood.
        v. Centrifuge the cells at 100g for 7 minutes to obtain a soft cell pellet.
    b. For T75 flask with attached cells:
        i. Wash cells with 5mls of 1X PBS solution Cat# P1408-013, remove 100% 1X PBS solution.
        ii. Add 5 ml of 1X Trypsin EDTA Cat# T1509-014, and incubate at room temperature for 2- 3 minutes.
        iii. At the end of trypsin incubation, add equal (5mL) volume of media with serum to neutralize the trypsin.
        iv. Transfer the cell suspension into sterile 15 ml conical tube in tissue culture hood.
        v. Centrifuge the cells at 100g for 7 minutes to obtain a soft cell pellet.
3. Reconstitute the soft cell pellet in 5 ml of Colony Forming Media #500 for T25 pre-coated flask with ECM, and 10 ml for T75 flask. Incubate the cells in this media for 24 hours.

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4. After incubation of the cells in Colony Forming Media #500 for 24 hours, remove 100% media and add fresh pre-warmed Colony Forming Media #250. Incubate the cells in Colony Forming Media #250 at $37^0$C humidified incubator with 5% carbon dioxide for 24 hours.
5. After incubation of the cells in Colony Forming Media #250 for 24 hours, remove 100% media and add fresh pre-warmed Colony Forming Media #100. Incubate the cells in Colony Forming Media #100 at $37^0$C humidified incubator with 5% carbon dioxide for 24 hours.
6. After incubation of the cells in Colony Forming Media #100 for 24 hours, remove 100% media and add fresh pre-warmed Serum Free Media. Incubate the cells in Colony Forming Media serum free at $37^0$C humidified incubator with 5% carbon dioxide.
7. After 48 hours in serum free media, the cells will start forming colonies. Within a week in a serum free condition the cells will form colonies.
8. The cells can be sub-cultured in serum free media using flasks pre-coated with ECM.