Feeding Guidelines

Supplies:

- Media (5 mL for T25, 15 mL for T75)
- Pipettes
- 15 mL or 50 mL conical tubes
- Optional: PBS

Steps:

1. Calculate the total volume of media you will need to feed all cells (ex. 35 mL for two T75 flasks and one T25 flask). Transfer appropriate volume of complete media to sterile conical tube under sterile conditions. This is to avoid re-warming the entire bottle of media multiple times.
2. Warm the aliquoted media in 37 degC water bath (will take 5-30 min depending on volume).
3. *Optional*: You can also warm an aliquot of PBS if you feel your cells need to be washed. Often require washing the first time cells are fed after seeding/harvest due to presence of RBCs and debris. If culture appears pretty clean, no need to wash.
4. Remove each flask requiring feeding from incubator and view each flask under microscope to determine percent confluence. Only remove and handle 1 flask at a time to avoid contamination.
5. Once media is up to temperature, move all flasks requiring feeding and warmed media to hood.
6. Perform each of the following steps for a single flask, prior to moving on to the next flask, to avoid contamination:
    a. Carefully remove spent media with sterile pipette.
    b. Optional: add 5 mL warmed PBS per 25 cm^2 to flask, and gently rock flask back and forth. Remove PBS.
    c. Add 5 mL fresh, pre-warmed complete growth media per 25 cm^2 of surface area.
7. Return flasks to incubator.