## TRYPsinIZATION OF ADHERENT CELLS

<table>
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<th>Step</th>
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| 1    | - Observe adherent cells microscopically  
      - Shake flask gently on the platform to assess adherence  
      - If too many cells are floating, it is an indication of low viability |
| 2    | - Discard medium into a waste beaker (spray beaker with ethanol before introducing into hood)  
      - Wash x2 with PBS ~3 mL each wash. Note: PBS does not contain Calcium/Magnesium to minimize cell sticking  
      - Discard PBS in waste flask |
| 3    | - Add 3 mL of trypsin/EDTA solution. Ensure trypsin covers the entire surface on which cells are adherent  
      - Allow 2-3 minutes for trypsin to work  
      - Trypsinization time varies depending on the cell type  
      - If detachment is low, place flask in incubator and inspect microscopically every 5 minutes  
      - Neuro2A cells detach easily at room temperature, 2-3 minutes is sufficient  
      - RAW cells take longer, ~10 minutes  
      - To facilitate detachment, ‘bang’ flask on a hard surface. Be careful not to break flask |
| 4    | - If ~ 50% of cells are floating, terminate reaction with ~ 3 mL medium containing FBS.  
      - FBS contains alpha-1-antitrypsin which inhibits trypsin  
      - Note: Excessive trypsinization kills the cells |
| 5    | - Aspirate cells with a 10 mL pipette  
      - Place them in a 15 mL conical tube  
      - Make sure tube is airtight before removing it from the hood |
| 6    | - Centrifuge at 200g for 5 minutes  
      - Room temperature is acceptable  
      - Brakes (acceleration/deceleration) can be applied |
| 7    | - Return 15 mL tube in the hood  
      - Carefully discard supernatant in the waste beaker in the hood  
      - Resuspend using PBS with 1 mL pipette. Medium can be used, however PBS results in less cell sticking  
      - Ensure disintegration of pellet via repetitive pipetting  
      - Small resuspension volume facilitates pellet disintegration |
| 8    | - Carry out a cell count/viability and sub-culture  
      - Typically 200,000 cells/mL, 10 mL for a 25 cm² flask |