**Supplies**

* Ice bucket filled with ice
* 5 x 30-50 mL glass beakers (plastic doesn’t work for beakers A and B)
* 1 x 400 mL Waste beaker
* 1 x 15 mL pre-washed Falcon test tube  
  (You will also need an additional tube filled with 10.5 ml water to balance the centrifuge - this balance tube should be left near the centrifuge.)
* 20 mL of Double-Strength Relax
* 2 mL of 0.8 mM Leupeptin
* 200 uL of 100 mM PMSF solution
* 17.0 mL of D.I. H2O
* 1 mL of 10% Triton
* 30 mL Single-Strength Relax (minimum)
* Plastic transfer pipettes
* Paper Towels

**For Human tissue only, also need:**

* 100% ethanol
* Decon solution
* Conflikt spray

**Part 1: Preparation**

1. Label the 5 beakers A, B, Wash, Triton, Relax
2. Push the beakers down into the ice so that about 25% of the beaker is embedded in the ice. Be careful not to get ice into the beakers as you do this.
3. Decide whether you are trying to get publication quality data or whether you are practicing. If you are not sure, ask Ken.

* If you are trying to get publication quality data, fill the first 4 beakers with the following solutions

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Beaker | DS Relax | Leupeptin Sol | PMSF Sol | Triton | H20 |
| A | 5 mL | 500 uL | 50 uL |  | 4.45 mL |
| B | 5 mL | 500 uL | 50 uL |  | 4.45 mL |
| Wash | 5 mL | 500 uL | 50 uL |  | 4.45 mL |
| Triton | 5 mL | 500 uL | 50 uL | 1mL | 3.45 mL |

* If you are learning the technique (practicing) fill the first 4 beakers with the following solutions instead (because leupeptin and PMSF are expensive, and we don’t need to waste these valuable chemicals when you are developing your skills.)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Beaker | DS Relax | Leupeptin Sol | PMSF Sol | Triton | H20 |
| A | 5 mL |  |  |  | 5 mL |
| B | 5 mL |  |  |  | 5 mL |
| Wash | 5 mL |  |  |  | 5 mL |
| Triton | 5 mL |  |  | 1mL | 4 mL |

The next steps need to be performed in quick succession. You don’t have time to delay and/or adjust your plans once you start. Read the protocol carefully in advance and make sure that you know exactly what you are trying to do. If you have questions, read the protocol again, and then again. If you still have questions, or if things are unclear, ask Ken.

**Part 2: Homogenization**

1. Set up your beakers and solutions next to the Polytron homogenizer on the short bench in MS533
2. Place folded paper towel under the homogenizer to catch drips.
3. Put into beaker A, one of
   * 1. Half rat heart
     2. Entire mouse heart
     3. Chunk of human tissue (~200 mg)
4. Place beaker A under homogenizer such that the blades touch the bottom of the glass beaker, and the tissue sample is directly in between the blades.
5. Blend for 1 to 3 seconds. (Optimum is 2 seconds. Count one Mississippi, two Mississippi and stop the griding. Alternatively, switch it on, let the blades reach full speed, and switch off, keeping the beaker under the blades until they come to rest). Take the beaker out and observe if the color of the solution has changed to reddish or if the muscle size has decreased. If not, repeat this step. If yes, then move to the next step.
6. Place Beaker A back on ice.
7. If a small piece of muscle has been left in Beaker A, transfer it to Beaker B.
8. Place Beaker B into the blender and blend for 1 to 2 seconds.
9. Place Beaker B back onto the ice.
10. Pour the contents of Beaker A into the falcon tube and cap the falcon tube.
11. Centrifuge the falcon tube using the Eppendorf Centrifuge located next to the blender (place the tube across from another falcon tube with an equal amount of liquid to balance). Centrifuge for 1 minute at 4°C & 1,200 rcf.
12. Take the falcon tube and carefully decant the solution in the Waste beaker.
13. Pour the contents of Beaker B into the falcon tube and cap the tube.
14. Disrupt the pellet with a clean dropper and then…
    * 1. Centrifuge - 1 minute at 4°C & 1,200 rcf.
      2. Pipette off supernatant
15. Add the contents of the Wash beaker
16. Disrupt the pellet with clean dropper and then…
    * 1. Centrifuge - 1 minute at 4°C & 1,200 rcf.
      2. Pipette off supernatant
17. Disrupt the pellet and pour the contents of the Triton beaker into the falcon tube
18. Place the falcon tube horizontally on the shaker (aka belly dancer) and leave for 30 ± 2 minutes to slowly oscillate at room temperature.

NOTE: do not discard ice yet; still needed for the beakers in part 3.

**Part 3: Removing Triton**

* 1. Remove the falcon tube from the horizontal shaker
  2. Centrifuge as previously - 1 minute at 4°C & 1,200 rcf.
  3. Pipette of supernatant without disrupting pellet
  4. Add 10 mL of Relax
  5. Disrupt pellet
  6. Repeat steps 2 to 4 at least twice
  7. Check that there are no bubbles at the top of the relax solution.
  8. If bubbles are present, repeat the rinse with more relax

1. If there are no bubbles, congratulations – you have successfully isolated chemically permeabilized cells.
2. Resuspend with 10mL of Relax as final step (individual fibers will be selected out of this suspension).

**Part 4: Cleanup**

1. Polytron Blender Blades:
   * 1. Pour a small amount of 100% Ethanol into a beaker.
     2. Place under blender blades and turn on blender for 2-3 seconds.
     3. Allow blender to come to a full stop and visually check blades for residual tissue pieces.
     4. If necessary, unplug blender from wall and remove residual tissue with forceps
     5. Repeat rinse in ethanol
     6. Allow to air-dry.
2. When working with animal tissue:
   * 1. Wash all non-disposable equipment as normal.
3. When working with human tissue:
   * 1. Clean all surfaces that have come in contact with human samples with Conflict Decon spray.
     2. Empty waste supernatant into a 250 mL beaker with Ci-Decon solution (disinfectant 1:128 concentrate)
     3. Empty down sink after 24 hours
     4. Place disposable pipettes, beakers etc. in an orange biohazard bag which should then be autoclaved
     5. Soak all glassware and dissecting tools in Ci-Decon and then wash them as usual after 24 hours.