# Preparation of Tonsil Lysate

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## Purpose

The protocol describes how to prepare human tonsil lysate for use in purification of ICAM-1, LFA-1, and PNAd.

## Materials

- Safety Equipments
  Lab Coat
  Latex Gloves
  Face Mask
  Bench Paper
- Fresh human tonsil tissue
- Parafilm
- Scissors
- Stainless steel mesh screen ( 200 mesh=74 μm, Type 316; Tylinter, Mentor, Ohio)
- RPMI-1640 medium
- Triton X-100
- Lysis Buffer:
  50 mM Tris-HCl, pH 7.5
  150 mM NaCl
  0.02% NaN3
  10 μg/ml Aprotinin
  10 μg/ml Pepstatin
  10 μg/ml Leupeptin
  1 mM PMSF (Made fresh the day of experiment. Dissolve in 100% EtOH)
  1 mM Benzamidine
  5 mM Iodoacetamide
- Whatman Paper
- Centrifuge Bottles
- Funnel
- Squeeze bottle
- Several Beakers
- Homogenizer (Fisher PowerGen 125)
- 50ml Corning tubes
- Ice Bucket & Ice
- Dounce homogenizer (40ml Pyrex Cat No. 7727-40)
- 1 liter bottle
- Stir bar
- 2 Side-arm flasks

## Procedure

*All work must be done at 4ºC.*

1. Collect 50 grams of tonsil tissue. (this should yield a large amount of ICAM-1, LFA-1, and PNAd)
   Note 1: Collecting this much tonsils can take some time. So it is good to collect from more than one source.
   Note 2: You can prep each sample as you receive it or wait until you have a ~ 50g of tonsils.
   - If you decide to prep them right away then perform steps 2 to 4 and then freeze at -80ºC.
   - If you want to wait until you have ~50g of tonsil, you should immediately freeze tonsils at -80ºC.
2. Clear a work area in the 4ºC walk-in fridge. Lay down bench paper and assemble all you materials.
3. Mince the tonsils with the scissors. Try and make the pieces a small as possible.
   Note: You can use a small glass plate as a cutting surface to make the job easier.
4. Fold the wire mesh in the shape of a cone and place in a funnel. Place funnel on the mouth of the beaker, to collect RPMI-1640.
5. Pour RPMI-1640 into a squeeze bottle. Wash the minces tonsils with copious amounts of RPMI-1640. Wash a small amount of tonsils at a time.
   (At this point you can freeze the tonsils until you have enough)
6. Set up the homogenizer with the large probe (1cm diameter).
7. Put a small amount of minced tonsil in the 50ml Corning tube and add lysis buffer to ~10ml mark.
8. Insert probe to the tube and the cover the neck of the tube and the main body of the probe with parafilm. This is very important. This prevents aerosols from escaping during the homogenizing process.
9. Homogenize in small burst to avoid heating the sample.
   Note 1: Homogenize till you liquefy the sample. You may still see a little bit of white strands, but these are connective tissue and will not completely break down.
   Note 2 : The probe will get clogged with connective tissue. Clean it of with tissue paper and rinse in with water by pulsing the probe to remove the rest of the stuck connective tissue. Once clean again you can continue homogenizing your sample.
10. Collect the homogenized tissue in a beaker that is kept on ice.
11. Using the dounce homogenizer, homogenize the collected sample using an up & down and left & right hand motion. Do this at least 10 times to get a very fine sample.
    Note: To avoid spilling you sample, don't use more than the volume stated on the side of the dounce homogenizer.
12. When you are done transfer homogenized tissue to a 1 liter bottle and add in the stir bar.
13. Bring the volume up to 1 liter by adding more of the Lysis buffer.
14. Add Triton-X 100 to a final concentration of 2% by volume.
    Note: The Triton-X-100 will not dissolve immediately. It will go into solution slowly over night.
15. Stir gently over night in the 4ºC walk-in.
16. Transfer the liquid to centrifuge bottles and spin down lysate at 8000g for 1 hour.
17. Decant lysate in a clean beaker. Discard pellet.
18. Filter lysate twice, using Buckner Funnel and Whatman paper as follows:
    - Cut the Whatman paper to cover the bottom of the Buckner funnel.

- Place funnel on side-arm flask and attach.
- Attach the first flask to the second and to the vacuum.
- Filter the lysate through the Whatman, changing it often as it gets clogged.

19. Filtrate may now be stored a 4ºC until it is time to run over an affinity column.

    Note: Don't store for more than a couple of days at 4ºC.

## References

K.D. Puri, E.B. Finger, G. Gaudernack, and T.A. Springer (1995). Sialomucin CD34 is the major L-selectin ligand in human tonsil high endothelial venules. *J Cell Biol* 131:261-270.