1X RIPA Lysis Buffer
Protocol

I. Procedure for Lysis of Adherent Cells

1. Remove growth medium from the cells by decanting or aspirating the medium.
2. Wash the cells with sterile PBS to remove residual medium. Slowly add a volume of sterile PBS equal to the original medium volume, being careful not to dislodge the cells. Mix gently and remove the wash solution. Repeat the wash once in order to remove any other minor contaminants. More washing steps can be done but two is usually sufficient to remove most of the contaminants.
3. After removal of the final wash solution from the cells, add an appropriate volume of RIPA Lysis Buffer (1 mL for 0.5 to 5 x 10^7 cells). Incubate on ice or in a refrigerator (2°–8°C) for 5 minutes.
4. Rapidly scrape the plate with a cell scraper to remove and lyse residual cells. Transfer the cell lysate to a tube on ice. The lysate can either be used immediately or quick-frozen in liquid nitrogen and stored at –70°C for future use. It is best to freeze the lysate before clarification since the freeze-thaw cycle may cause some proteins to denature and aggregate.
5. Clarify the lysate by centrifugation at 8,000 x g for 10 minutes at 4°C to pellet the cell debris.
   Note: If a mucoid aggregate of denatured nucleic acids is present, carefully remove it with a micropipette before centrifugation.
6. Carefully transfer the supernatant containing the soluble proteins to a tube on ice for immunoprecipitation or other analysis.

II. Procedure for Lysis of Suspension Cultured Cells

1. Pellet the cells by centrifugation at 450 x g for 5 minutes.
2. Carefully remove the medium from the cell pellet by decanting or aspirating the medium.
3. Wash the cells to remove residual medium. Add a volume of sterile PBS equal to the original medium volume. Mix or vortex briefly to resuspend the cells completely. Centrifuge for 5 minutes at 450 x g to pellet the cells and carefully remove wash solution supernatant. Repeat the wash once in order to remove any other minor contaminants. More washing steps can be done, but two is usually sufficient to remove most of the contaminants.
4. After removal of the final wash solution from the cells, add an appropriate volume of RIPA Lysis Buffer (1 mL for 0.5 to 5 x 10^7 cells). Mix or vortex briefly to resuspend the cells completely. Incubate on ice or in a refrigerator (2°–8°C) for 5 minutes. Vortex briefly to resuspend and lyse residual cells.
5. The lysate can either be used immediately or quick-frozen in liquid nitrogen and stored at –70°C for future use. It is best to freeze the lysate before clarification, since the freeze-thaw cycle may cause some proteins to denature and aggregate.
6. Clarify the lysate by centrifugation at 8,000 x g for 10 minutes at 4°C to pellet the cell debris.
   Note: If a mucoid aggregate of denatured nucleic acids is present, carefully remove it with a micropipette before centrifugation.
7. Carefully transfer the supernatant containing the soluble protein to a tube on ice for immunoprecipitation or other analysis.

References

Antibodies a Laboratory Manual, Harlow, E. and Lane, D., Cold Spring Harbor Press (Cold Spring Harbor, NY: 1988), p 449.