

Triton X-100 Solubility Assay

Variations of Protocols used in:

Parreno et al., IOVS 2020; PMID: 32492110

Parreno et al., J.Anat 2017; PMID: 27807861

Parreno et al., FEBS Lettr.; 2014 PMID: 25150168

Seed cells at 250,000 cells/well in a 6well dish

- After 24 hours transferred into serum-reduced media (0.5%FBS) overnight
- Treat cells with drug
- After a given amount of time, wash cell in PBS, once quickly
- 80uL of Cytoskeletal buffer* (no PFA) + 0.1%Triton (CT) was placed on cells and spread evenly on culture dish surface
- After ~2 minute incubation, solution was removed and 8uL of 10X RIPA was added for a total of 88uL solution (CTR) THIS is the soluble portion
- To harvest insoluble protions, the remaining cells on the culture dish were mixed with 88uL of CTR solution
- Run equal volumes of soluble and insoluble fractions on Western blot.

*Cytoskeletal Buffer:

100 mM NaCl, 3 mM MgCl₂, 300 mM sucrose, 1 mM EGTA, 10 mM PIPES, and pH 6.8