

Asp3 Substrates Test Using Western Blot Assay

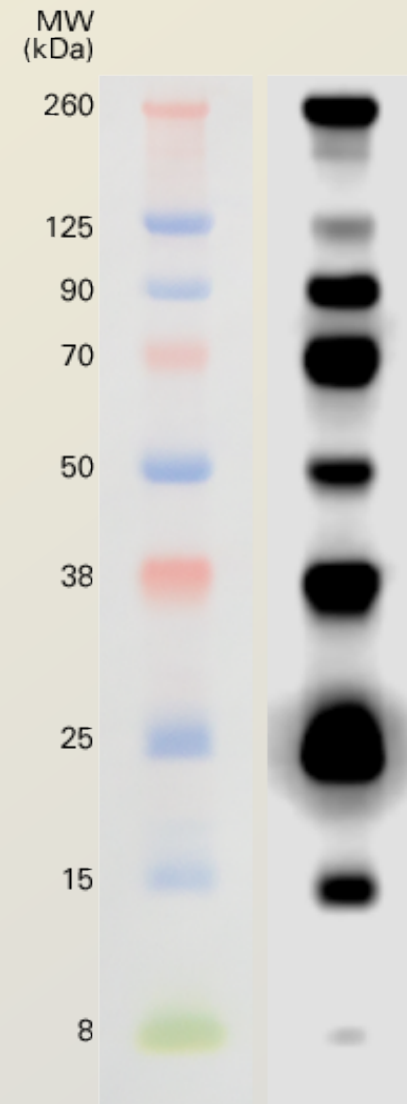
What Do We Know?

- Egress and invasion are impaired in Asp3 iKD
- Asp3 is an aspartic protease

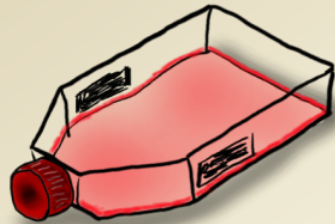
- What are the substrates of Asp3?
- Check proteins that are involved in egress and invasion using WB

Western Blot/ Protein Immunoblot

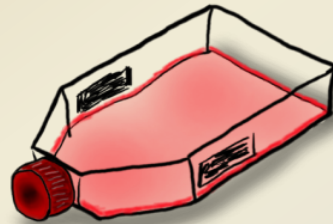
- Used to detect specific proteins in a sample of tissue /extract.
- It uses gel electrophoresis to separate proteins by denatured proteins



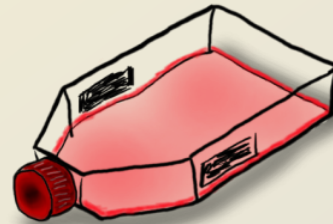
Protein Extraction



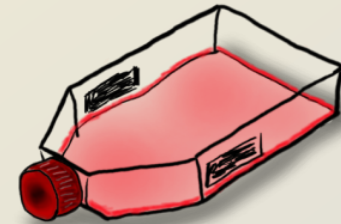
T. Gondii
WT
(+Asp3)



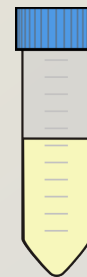
T. Gondii
-ATC
(+Asp3)

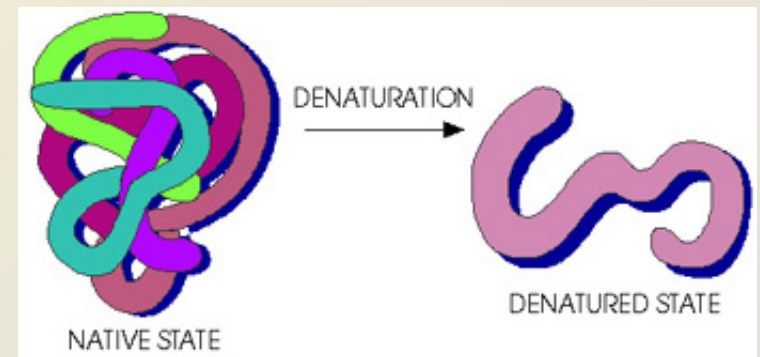
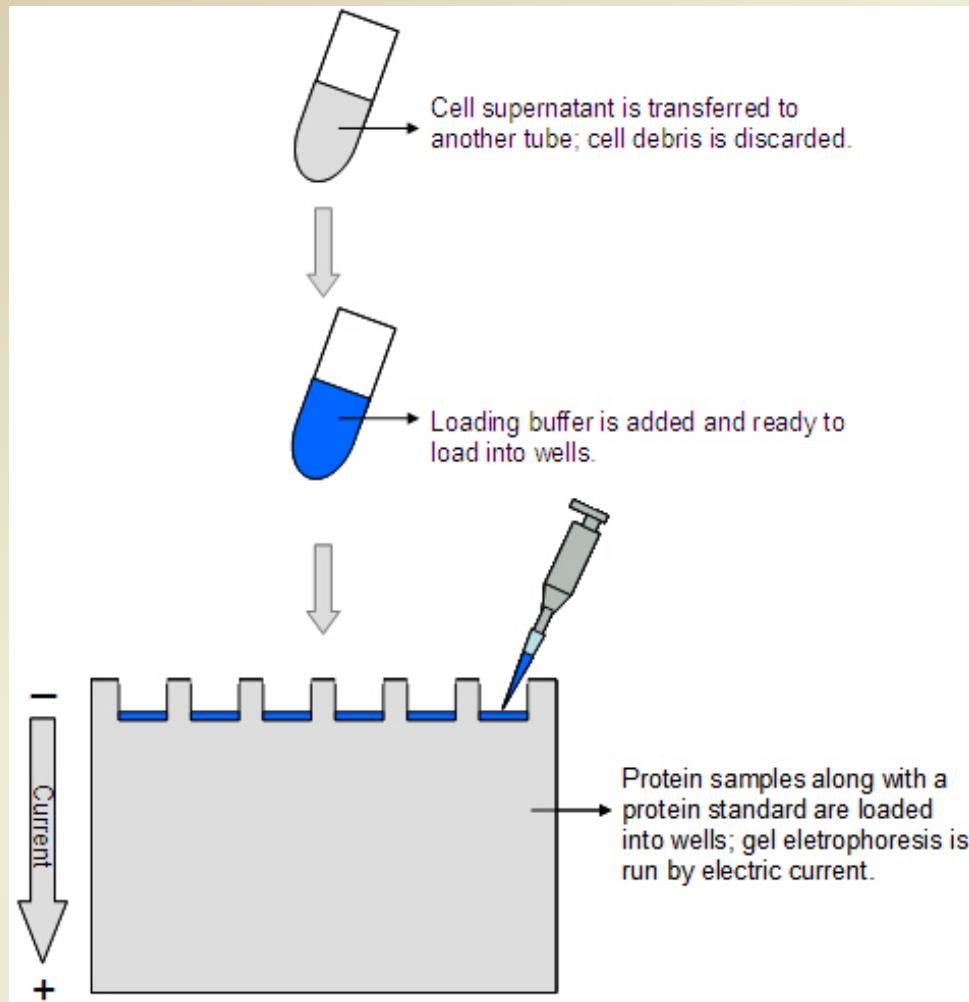


T. Gondii
+ATC 24hrs
(-Asp3)



T. Gondii
+ATC 48hr
(-Asp3)





SDS



Step 1
Perform
electrophoresis



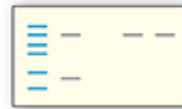
Step 2
Transfer



Step 3
Block with ScanLater
5X Blocking Buffer



Step 4
Incubate with
primary antibodies



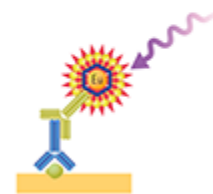
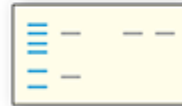
Rabbit anti-X
Mouse anti-Y
Streptavidin



Step 5
Wash with
ScanLater Wash Buffer



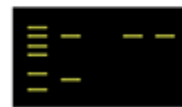
Step 6
Incubate with ScanLater
Secondary Antibody



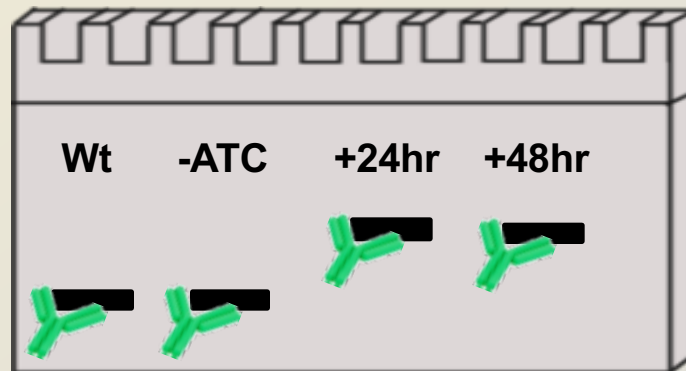
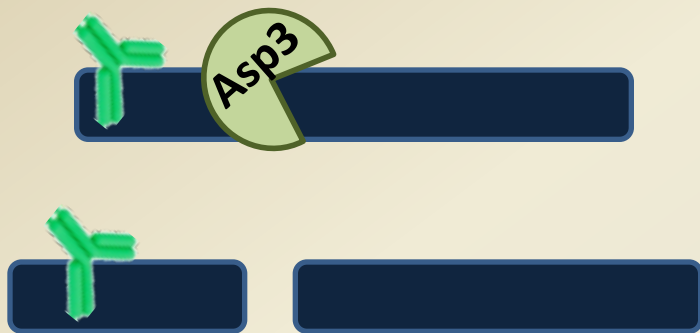
Step 7
Wash with ScanLater
Wash Buffer

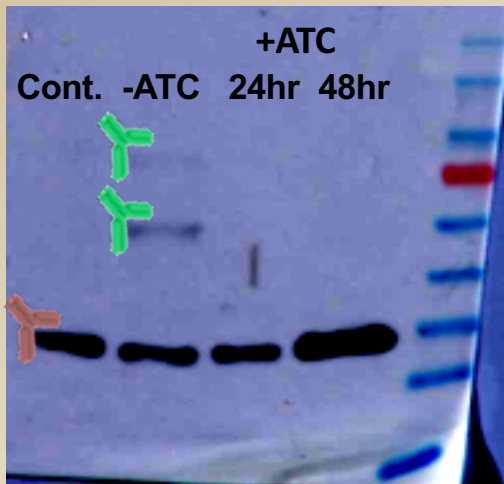



Step 8
Measure with
ScanLater Western Blot
SpectraMax Detection Cartridge




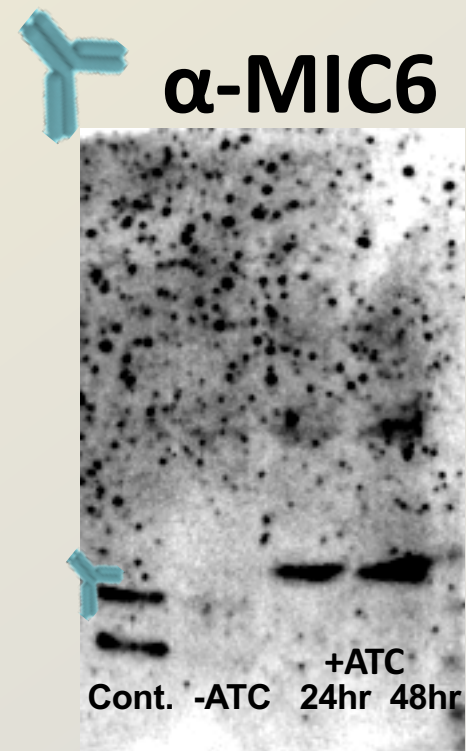
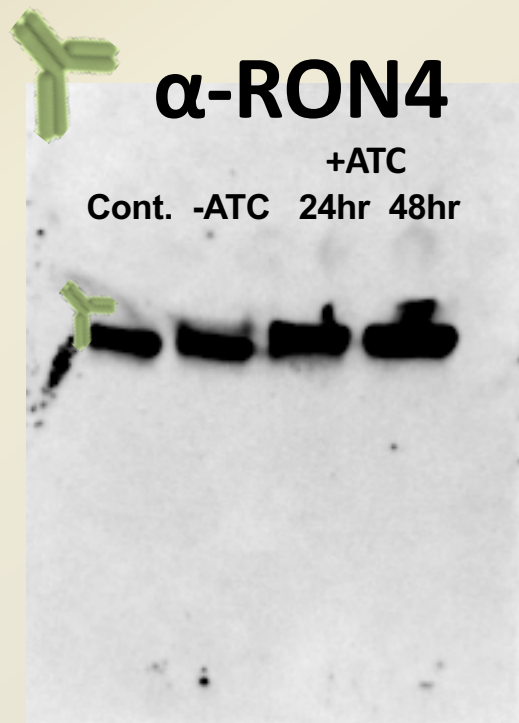
Results

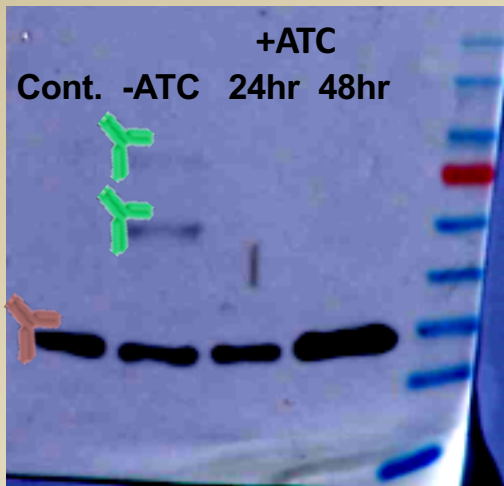






 α -Ty tag


 α -GRA3

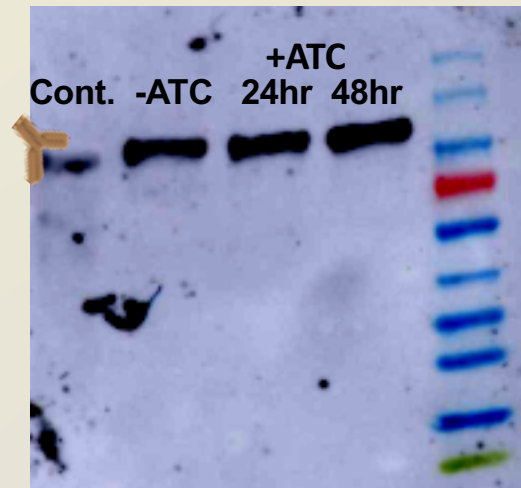


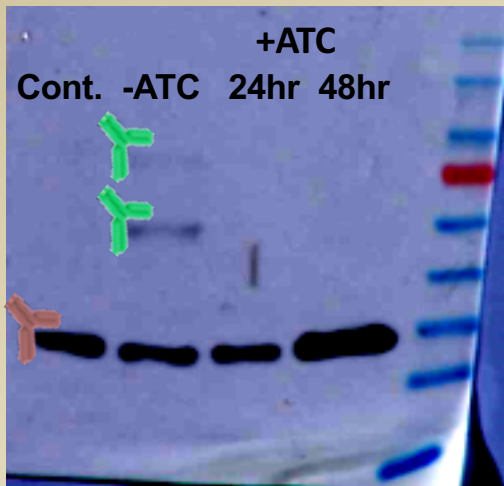



 α -Ty tag

 α -GRA3


 α -MIC2

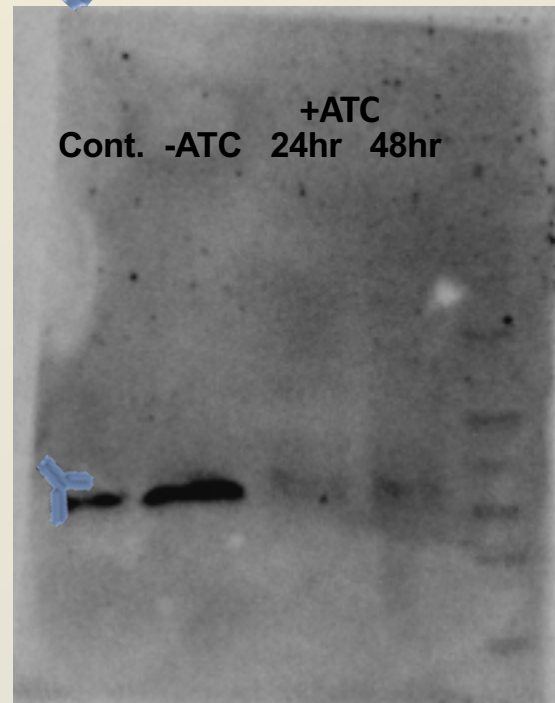




 α -Ty tag

 α -GRA3

 α -MIC3



Thank you!