

Sugden Lab.

### **LMP-1 Pulse Chase**

#### **<sup>35</sup>S Labeling of Cells for LMP-1**

- 1) Wash  $1 \times 10^7$  cells w/R10F lacking Met & Cys
- 2) Incubate 30min @ 37°C w/R10F (-Met, Cys)
- 3) Spin down and resuspend in 1ml R10F (-Met, Cys) w/250 $\mu$ Ci for 20 or 60 minutes.
- 4) Wash 1x in RPMI1640
- 5) Incubate in R10F for 0-24 hours
- 6) Harvest  $1 \times 10^7$  cells and wash 2x w/RPMI1640

#### **Immunoprecipitation of LMP-1**

- 1) Resuspend  $1 \times 10^7$  cells in 200 $\mu$ l 5xRIPA + 5x Protease inhibitor stock and sonicate.
- 2) Add 800 $\mu$ l H<sub>2</sub>O
- 3) Remove 500 $\mu$ l and boil for 10 minutes.
- 4) Add 350 $\mu$ l of Goat  $\alpha$ Rabbit immuno beads and incubate in cold room for 20min.
- 5) Spin 5min and wash 1x w/1xRIPA
- 6) Add 130 $\mu$ l  $\alpha$ LMP-1 serum and incubate 30minutes in cold room.
- 7) Add 150 $\mu$ l Goat  $\alpha$ Rabbit immuno beads and incubate 30minutes in cold room.
- 8) Wash 4x w/1xRIPA
- 9) Boil in 4x Sample buffer and run a 10% SDS PAGE
- 10) Quantitate on phosphorimager