

Protocols for immunization of mice

Extended ip protocol (Ext IP)

1. Overall Schedule

Immunize day 0, boost days 14, 28, 42; fuse day 46.

2. Antigen preparation

10 ug antigen is mixed with Imject alum (Pierce) plus 10 nmols CpG ODN 1826 (sequence TCCATGACGTTCCCTGACGTT) in a total volume of 100 uL for each immunization.

3. Immunizations

Each immunization and boost is administered i.p., and the spleen is collected aseptically 4 days after the last boost.

Footpad/hock protocol (Ftpd)

1. Overall Schedule

Immunize day 0 in footpad, boost day 12 in hock, harvest popliteal LN and fuse day 15.

2. Antigen preparation

For each mouse, 25 to 50 ug antigen in 12.5 uL PBS mixed with equal volume of TiterMax by vortexing until a stable emulsion is formed.

3. Immunization

A maximum of 25 uL of the antigen-adjuvant mixture is injected into one rear footpad of a mouse, and up to 50 uL of the mixture is injected into the hock area of same leg. Popliteal LN is harvested aseptically for fusion.

Extended ip, iv protocol (Ext IPIV)

1. Overall Schedule

Immunize day 0, boost days 14, 21, 28, 29, 30. Harvest spleen day 31.

2. Antigen preparation

For the initial immunization (Day 0) 100 ug of antigen in PBS is mixed with an equal volume of RIBI adjuvant (or Sigma Adjuvant system). For the boosts on days 14, and 21, 50 ug of antigen in PBS is mixed with an equal volume of RIBI adjuvant. For boosts on days 28, 29, and 30, 50 ug is injected in PBS without mixing with adjuvant.

3. Immunization

The initial immunization (Day 0) and the first two boosts (Days 14, 21) are given i.p. The last three boosts (Days 28, 29, 30) are given i.v. in the tail. The spleen is harvested aseptically on day 31.