

SNPRC Immunology Core Laboratory
Elution of analytes from Vaginal Swabs

Materials:

Buffers: PBS-T (1X PBS / 0.05% Tween 20)

PBS-T/HALT (PBS-T + 1X HALT)

HALT Protease Inhibitor Cocktail (100X)-Thermo Scientific/Pierce

Procedure:

1. For elution of vaginal secretions from the sponges use UltraFree MC DV Durapore-PVDF (Millipore, cat. no. UFC30DV00). Pre-wet filter membrane with 50 μ l of Buffer (PBS-T) and incubate 10 minutes with shaking at 1000 rpm, room temperature. Spin tubes for 5 minutes at 10,000 g.
2. Eliminate buffer from tube using vacuum and sterile tip.
3. Place the sponge in 250 μ l of extraction buffer (PBS-T/HALT) in the upper chamber of the UltraFree MC DV Durapore-PVDF tube. Incubate tubes for 60 minutes at 4°C with shaking.
Note: cut the sponge shaft with scissors while the sponge is resting in the upper chamber of the tube.
4. Spin Durapore tube at high speed (10,000 x g for 10 minutes) at 4°C.
5. Estimate the volume in the lower chamber, aliquot the eluted buffer in 2 tubes, and placed at -80°C storage.
6. Because the volume of sample collected could not be standardized, all cytokine values will be corrected using a previously reported dilution factor $\{[(x - y) + 0.2625]/(x - y)\}$, where **x** is the weight of sponge with sample (in grams), **y** is the weight of the dry sponge, and 0.2625 is the weight of 250 μ l of buffer added for elution}.