

# Biotech Trading Partners

## Multiplexing SPOT Assays

Make the working dilution of the Specimen Diluent/Wash Buffer by bringing one bottle of the 20X Specimen Diluent/Wash Buffer Concentrate to 500ml with distilled water. Add the entire contents of one bottle of the Blocking Buffer. **MIX WELL**

Prepare 1:101 dilution of the specimens as follows:

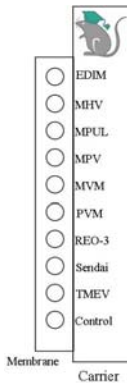
**1**

Add 2ml of diluted Specimen Diluent/Wash Buffer made in step one to a provided test tube. Label tube for identification.

Add 20µl of each specimen to the appropriate tube. **MIX WELL**

Without touching the membrane, remove the required number of SMART-SPOT devices and reseal the pouch. Using a sharpie, label the carrier portion with your specimen ID number.

**2**



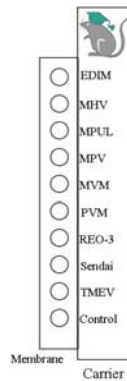
Place the SMART-SPOT Device into an incubation tray channel

Using the included transfer pipettes, add all of the diluted specimen to the appropriate tray channels. Gently rock the tray several times by hand to mix.

Incubate for 30 Minutes at RT

WASH as per package insert instructions

**3**



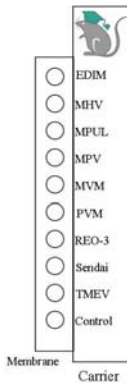
Add 2ml Conjugate-1 to all channels with a device.

Gently rock the tray several times by hand to mix

Incubate for 20 Minutes at RT

WASH as per package insert instructions

**4**



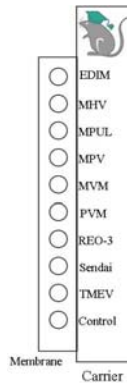
Add 2ml Conjugate-2 to all channels with a device

Gently rock the tray several times by hand to mix

Incubate for 10 Minutes at RT

WASH as per package insert instructions

**5**



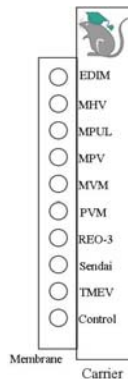
Add 2ml Chromogen to all channels with a device

Gently rock the tray several times by hand to mix

Incubate for 5 Minutes at RT

WASH as per package insert instructions

**6**



Remove the SMART-SPOT Device and rinse with the working dilution of the diluents/wash buffer to stop the reaction

Place the device on a paper towel to dry

Read visually within 48 hours



The Mouse Basic Panel is used in this illustration, but all SPOT assays are performed identically

