DATA SHEET

# SMART-SPOT® Sentinel Panel Test

## Catalog # SMART-SPT8R, 8-Analyte Basic Screen Panel
## For the detection of antibodies to 8 antigens in Rat Sera
## For Use On Research Animals Only

### ASSAY PRINCIPLE

The **SMART-SPOT** device consists of a membrane mounted to a hard carrier. The membrane is the reactive unit and should not be touched. The membrane has 9 invisible test "spots". Each of the first 8 spots is a specific antigen, as indicated in the diagram below, and the 9th spot is a Test Control. The Test Control spot insures that a specimen was added and that all of the reagents worked properly.

During the first incubation with the diluted specimen, any antibody that is reactive with the specific antigen will bind to the spot. After washing to remove the rest of the sample, Enzyme Conjugate-1 is added. After another series of washes, Enzyme Conjugate-2 is added. If antibodies have been bound to the spots, the Enzyme Conjugates will then bind to these antibodies. After another series of washes, a Chromogen is added. If the Enzyme Conjugates have bound to a spot, the Chromogen will turn the spot from invisible to a shade of purple. The strip is rinsed to stop the reaction and any purple color in an antigen spot is indicative of antibody present to that specific antigen. The Test Control spot MUST be positive (a shade of purple) in order for the test to be valid.

The SMART-SPT8R assay tests for the following:

**Position 1: KRV** (Kilham Rat Virus)

**Position 2: MPUL** (Mycoplasma pulmonis)

**Position 3: PVM** (Pneumonia Virus of Mice)

**Position 4: RCV/SDAV** (Rat Coronavirus/Sialodacroadenitis Virus)

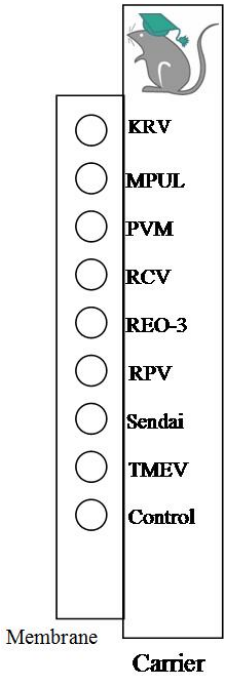
**Position 5: REO-3** (Respiratory Enteric Orphan Virus)

**Position 6: RPV, *r*-VP2** (Mouse Parvovirus)

**Position 7: Sendai** (Sendai Virus)

**Position 8: TMEV(GD7)** (Theiler's Murine Encephalomyelitis irus)

**Position 9: Test Control**



## REAGENTS PROVIDED

| | | |
|---|---|---|
| Test Strips: …… | **Eight (8)** | **SMART-SP●T** devices |
| **Enzyme Conjugate -1**: …… | **One (1)** | 20ml bottle of anti-rat IgG conjugate-1 |
| **Enzyme Conjugate -2**: …… | **One (1)** | 20ml bottle of anti-rat IgG conjugate-2 |
| **Chromogen**: …… | **One (1)** | 20ml bottle of substrate |
| Blocking Buffer: …… | **Three (3)** | Bottles of powdered blocking buffer |
| Specimen Diluent/Wash Buffer Concentrate: | **Three (3)** | 25ml bottles of concentrated (20X) buffer |
| Incubation Tray: …… | **One (1)** | 8-channel incubation tray |
| Test Tubes: …… | **Eight (8)** | Specimen dilution tubes |
| Transfer Pipettes: …… | **Eight (8)** | Diluted specimen transfer pipettes |

## MATERIALS REQUIRED BUT NOT PROVIDED

Pipettes capable of delivering 20µl and 2ml
Test tube rack to hold the diluted specimens
Squeeze bottle for washing strips
Distilled or reagent grade water

## REAGENT STORAGE

Store the kit reagents and **SMART-SP●T** devices strips between 2 - 8º C.
Store the diluted Specimen Diluent/Wash Buffer between 2 - 8º C. This diluted buffer is good for up to 10 days. Do not add fresh buffer to old buffer.

## SERUM COLLECTION AND HANDLING

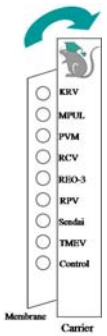
This test utilizes the specimen's serum: coagulate the blood and remove the serum. The use of "bloody" sera is contra-indicated. Serum samples should be refrigerated as soon as possible after collection and tested within 48 hours. If the specimen is not to be tested within 48 hours after collection, the serum sample should be stored at 0°C or lower.
Do not heat-inactivate serum and avoid repeated freezing and thawing of samples.
Vortex (mix well) all samples before using.
**Do not use pooled specimens as this adversely will affect the performance of the assay.**

Test samples are diluted 1:101 in the Specimen Diluent/Wash Buffer (20µl of sera + 2ml of Specimen Diluent/Wash Buffer)
**All Reagents must be at room temperature before beginning the assay.**

## PROCEDURAL NOTES

Allow all reagents and samples to come to room temperature before testing. It is normal for the concentrated Diluent/Wash Buffer to crystallize when cold. The crystals will re-dissolve once the solution returns to room temperature.
Do not use reagents beyond the expiration date printed on the label.
Do not reuse provided test tubes, transfer pipettes, or channels in the incubation tray or the **SMART-SP●T** device.
Do not touch the membrane portion of the **SMART-SP●T** device
Do not inter-mix conjugates between different kits or different lot numbers of the same kit: these components are balanced to work together as a unit. The chromogen, blocking buffer, and Specimen Diluent/Wash Buffer are universal reagents and can be inter-changed between all **SMART-SP●T** kits.

The **SMART-SP●T** devices are shipped flat and must be "bent" into the working configuration at the time of use. Place the device on a dry flat surface and gently bend the carrier up so that the mouse is on the top left side. The membrane will sit flat against the incubation tray floor and the carrier will be straight up in the incubation channel to hold the device in place.



## PROCEDURE

See attached procedure guide. All procedures and reagents are at room temperature (15 – 25° C).

## TROUBLESHOOTING

If the Test Control spot is not visible the test is not valid. Recheck your procedure and insure that all reagents are at room temperature before starting the assay.

If all the spots have visible color or there is a high degree of background staining usually indicates incomplete washing. Washing is extremely important in all assays, and incomplete washing leaves behind excess reagents that may give false positive results. Also check the three "T's": Time, Temperature and Technique. Time: insure that the timing on the incubation stages is adhered to. Temperature: temperatures above 25°C may adversely affect the assay; Technique: check all pipettes to insure that they are properly delivering the correct volume to produce a 1:101 dilution of the specimens.

## READING RESULTS

Allow the test membrane to dry for at least 30 minutes before reading and should be read within 48 hours after the start of the drying period. Drying time is dependent upon lab conditions (temperature and humidity). The Test Control spot must be visible for the test to be valid. Any purple color in a specific antigen spot is indicative of antibody present to that specific antigen and further testing is required by an alternate method.

## EXPECTED VALUES

The normal value is Negative. Studies have shown that antibodies may take up to 21 days to appear after exposure; therefore, negative specimen results should be reviewed in relation to a possible exposure date. SMART-SPOT is designed as a screening assay and positive specimen results should be confirmed by an alternate method. Although uncommon, false positive results may occur from non-specific antibodies binding to the media in which the antigen is derived.

This product is warranted to perform as described in the labeling provided that: the product is stored and used as directed; used before the expiration dating; and adequate quality control is performed. No other warranty is implied, nor are we liable for any consequential damages arising out of the aforesaid warranty.

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# SMART-SPOT® PROCEDURE GUIDE

***8-Analyte Panel (Rat Sera), Catalog #SMART-SPT8R***

### *INITIAL SETUP*

1. 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**
2. Prepare 1:101 dilution of the specimens as follows:
   Add 2ml of diluted Specimen Diluent/Wash Buffer made in step one to a provided test tube
   Add 20µl of each specimen to the appropriate tube. **MIX WELL**

### *SERUM INCUBATION STAGE*

3. Without touching the membrane, remove the required number of SMART-SPOT devices and reseal the pouch. (Unused devices must be kept sealed in a dry environment) The working configuration of the device is that the membrane is at a $90^0$ angle to the carrier. The membrane sits flat on the floor of the incubation channel and the carrier sits up straight in the channel and holds the device in place. Using a sharpie, label the carrier portion with your specimen ID number.
4. Insert the SMART-SPOT device into the appropriate channel in the incubation tray insuring that the membrane is against the floor of the channel and that the carrier is securely wedged into the channel.
5. Using the provided transfer pipettes, transfer the all of the diluted specimen from step 1 into the appropriate channel in the incubation tray. Rock the tray several times to mix and insure that the membrane is fully covered by the diluted specimen.
6. Incubate at room temperature for **30 MINUTES.**

### *WASH STAGE*

7. After 30 minutes, pick up the tray and rest it against the palm of your hand with your thumb gently crossing over the top of the device carriers. Invert the tray and dump (the carrier will hold the device in place). Wash the strip by directing the stream of the working dilution of the Specimen Diluent/Wash Buffer directly along the strip and completely fill each channel to the top. Dump again.
8. Repeat the above step four more times for a total of **5 WASHES.**
9. After the last wash, shake the tray 5-6 times to remove excess liquid.

### *CONJUGATE-1 INCUBATION STAGE*

10. Add 2ml of CONJUGATE-1 to each channel with a device and rock the tray several times to mix and insure that the membrane is fully covered.
11. Incubate at room temperature for **20 MINUTES.**

### *WASH STAGE*

12. After 20 minutes, pick up the tray and rest it against the palm of your hand with your thumb gently crossing over the top of the device carriers. Invert the tray and dump (the carrier will hold the device in place). Wash the strip by directing the stream of the working dilution of the Specimen Diluent/Wash Buffer directly along the strip and completely fill each channel to the top. Dump again.
13. Repeat the above step four more times for a total of **5 WASHES.**
14. After the last wash, shake the tray 5-6 times to remove excess liquid.

### *CONJUGATE-2 INCUBATION STAGE*

15. Add 2ml of CONJUGATE-2 to each channel with a device and rock the tray several times to mix and insure that the membrane is fully covered.
16. Incubate at room temperature for **10 MINUTES.**

### *WASH STAGE*

17. After 10 minutes, pick up the tray and rest it against the palm of your hand with your thumb gently crossing over the top of the device carriers. Invert the tray and dump (the carrier will hold the device in place). Wash the strip by directing the stream of the working dilution of the Specimen Diluent/Wash Buffer directly along the strip and completely fill each channel to the top. Dump again.
18. Repeat the above step four more times for a total of **5 WASHES.**
19. After the last wash, shake the tray 5-6 times to remove excess liquid.

### *CHROMOGEN STAGE*

20. Add 2ml of CHROMOGEN to each channel with a device and rock the tray several times to mix and insure that the membrane is fully covered.
21. Incubate at room temperature for **5 MINUTES.**

### *STOP STAGE / DRYING STAGE*

22. After 5 minutes, pick up the tray and rest it against the palm of your hand with your thumb gently crossing over the top of the device carriers. Invert the tray and dump (the carrier will hold the device in place). Wash the strip by directing the stream of the working dilution of the Specimen Diluent/Wash Buffer directly along the strip and completely fill each channel to the top. Repeat one more time for a total of 2 washes.
23. Remove the device from the tray and place on a paper towel to dry for *at least* 30 minutes. The strips should read within 48 hours after the start of the drying period. Any purple color present on the specific spot is indicative of antibody present to that antigen and further testing is suggested.