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## Certificate of Analysis

### **IFA SPECIAL SERUM DILUTING BUFFER**

**CATALOG NO.:** SSDB-100ML

**VOLUME:** 100 ml

**LOT:** P120125-001

**EXPIRATION:** 27 March 2016

**DESCRIPTION:** Special Serum Diluting Buffer is PBS-0.09% sodium azide with 10% adult bovine serum added. Adult bovine serum is used in this diluting buffer instead of BSA because some vaccines used in canine, feline and porcine species contain bovine serum components which cause antibodies to be made that may give a high background fluorescence on IFA. Adding bovine serum to the diluent increases the globulin fraction sufficiently to block any antibody that might react with residual bovine globulin on the cells on the substrate slide, and thus reduce the background fluorescence.

**QUALITY CONTROL METHOD** Indirect FA using VMRD Positive Controls or antiserum, corresponding secondary conjugates and slides on one each of the following systems: Canine CCV (catalog no. PAB-CCV, CJ-F-PORG-AP-10ML, and SLD-IFA-CCV), Equine ERV (catalog no. PC-IFA-ERV-EQ, CJ-F-EQUG-10ML, and SLD-IFA-ERV), Feline FVR (catalog no. PC-IFA-FVR, CJ-F-FELG-10ML, and SLD-IFA-FVR), and Porcine TGE (catalog no. PC-IFA-TGE, CJ-F-PORG-AP-10ML, and SLD-IFA-TGE).

**Specific Reaction:** Results for this buffer lot give similar signals as the previous lot and the buffer alone was negative with no background.

**Comments:** NA

**INTENDED USE:** Special Serum Diluting Buffer should be used for diluting canine, equine, and feline serum for IFA. **Do not use for any bovine IFA testing.**

**STORAGE:** Store at 2-7°C.

FOR *IN VITRO* LABORATORY USE ONLY.

**WARRANTY:** VMRD, Inc. warrants that this product is as described in the quantity and contents stated on the label at the time of delivery to the customer. NO OTHER WARRANTIES, EXPRESS OR IMPLIED, ARE MADE BEYOND THE LABEL DESCRIPTION, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. Remedy is limited to replacement of the product or refund of the purchase price. VMRD, Inc. is not liable for property damage, personal injury, or economic loss caused by the product. The information listed in this information sheet is provided for reference only, and should not be substituted for the user's own incoming material quality control.

**RECOMMENDED STAINING PROCEDURE FOR INDIRECT FA:**

1. Warm slide to room temperature before removing from foil pouch.
2. Place diluted serum on the designated wells. Dilute serum in serum diluting buffer, pH 7.2 (catalog no. FASDB-100ML) however if high background due to anti-bovine IgG activity is present it may be advisable to use SSDB-100ML.
3. Incubate slide in humid chamber at 37°C for 30 minutes.
4. Using a wash bottle, gently rinse slide briefly in FA rinse buffer, pH 9.0 (catalog no. FARB-4X) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
5. Drain slide and dry around wells by pressing blotter (included in pouch) to front surface. Place labeled anti-IgG or IgM on the wells.
6. Incubate as in step 3.
7. Rinse as in step 4.
8. Drain slide and dry back and edges with a paper towel. Do not allow stained surface to dry. Do not rinse with water.
9. Mount with mounting fluid [glycerol/FA rinse buffer, pH 9.0, (50/50)] (catalog no. FAMF-10ML) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be made at 400X.

**RECOMMENDED STAINING PROCEDURE FOR DIRECT FA:**

1. Warm slide to room temperature before removing from foil pouch.
2. Place of direct FA conjugate on the designated wells.
3. Incubate slide in humid chamber at 37°C for 30 minutes.
4. Using a wash bottle, gently rinse slide briefly in FA rinse buffer, pH 9.0 (catalog no. FARB-4X) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
5. Drain slide and dry back and edges with a paper towel. Do not allow stained surface to dry. Do not rinse with water.
6. Mount with mounting fluid [glycerol/FA rinse buffer, pH 9.0, (50/50)] (catalog no. FAMF-10ML) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be at 400X.

**SERUM DILUTING BUFFER (pH 7.2):\***

- Na<sub>2</sub>HPO<sub>4</sub> . . . . . 1.19 gm
- NaH<sub>2</sub>PO<sub>4</sub> . . . . . 0.22 gm
- NaCl . . . . . 8.55 gm
- BSA . . . . . 10.0 gm
- DI/dH<sub>2</sub>O . . . . . Q.S. to 1 liter

\* This recipe makes 1 liter. If you need less, adjust recipe accordingly. Store at 2-7°C. Add 0.09% NaN<sub>3</sub> if diluted serum is not going to be used within one week.

**4X FA RINSE BUFFER (pH 9.0):**

- Na<sub>2</sub>CO<sub>3</sub> . . . . . 11.4 gm
- NaHCO<sub>3</sub> . . . . . 33.6 gm
- NaCl . . . . . 8.5 gm
- DI/dH<sub>2</sub>O . . . . . Q.S. to 1 liter

Final pH should be 9.0-9.5. This is a 4X concentrate and should be diluted 1/4 with DI/distilled water for use as a working buffer. Keep in a tightly stoppered container at room temperature. MOUNTING FLUID is made by mixing glycerol and FA rinse buffer, pH 9.0, in equal proportions.