# **Certificate of Analysis**

## FA CONJUGATE DILUTING BUFFER

CATALOG NO.: 210-91-CB

**VOLUME:** 100ml **LOT:** P090722-003

**EXPIRATION:** 14 July 2011

**DESCRIPTION:** Conjugate Diluting Buffer is PBS with 1% BSA, preserved with 0.09% sodium azide.

**QUALITY CONTROL METHOD:** Direct FA using 1:50 dilution of 50X Conjugates and the corresponding slides on each of the following VMRD systems: Bovine PI-3 (catalog no. CJ-F-PI3-50X, and SLD-IFA-PI3), Canine CDV (catalog no. CJ-F-CDV-50X, and SLD-IFA-CDV), Equine/Llama ERV (catalog no. CJ-F-ERV-50X, and SLD-IFA-ERV), Feline FVR (catalog no. CJ-F-FVR-50X, and SLD-IFA-PVV), and Porcine PPV (catalog no. CJ-F-PPV-50X, and SLD-IFA-PPV).

**Specific Reaction:** Each system produced at least a 3+ signal with no background at a 1:50

dilution.

**Comments:** NA

**INTENDED USE:** Conjugate Diluting Buffer should be used for diluting concentrated conjugates for direct and indirect fluorescence.

**STORAGE:** Store at 2-7°C.

#### FOR IN VITRO LABORATORY USE ONLY.

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#### RECOMMENDED STAINING PROCEDURE FOR INDIRECT FA:

- 1. Warm slide to room temperature before removing from foil pouch.
- 2. Place 50 μl diluted serum on the designated wells. Dilute serum in serum diluting buffer, pH 7.2 (catalog no. 210-93-SB).
- 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. 210-90-RB) 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 50  $\mu$ l 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 <u>back and edges</u> 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. 210-92-MF) 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 50 µl 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. 210-90-RB) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
- 5. Drain slide and dry <u>back and edges</u> 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. 210-92-MF) 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

<sup>\*</sup> 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>
-	NaCl
_	DI/dH <sub>2</sub> O O.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.