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## Certificate of Analysis

### **POWDERED FA RINSE BUFFER**

**CATALOG NO.:** FARB-4X

**VOLUME:** 1 pouch

**LOT:** P110201-005

**EXPIRATION:** 06 May 2013

**DESCRIPTION:** FA Rinse Buffer Powder, pH 9.0-9.5

**RECONSTITUTION AND STORAGE:** Dissolve in 1 liter of deionized/distilled water to make 4X concentrate. Dilute this concentrate 1/4 for final use. Powdered and reconstituted FA Rinse Buffer should be stored at room temperature.

**QUALITY CONTROL METHOD:** Reconstitute in 1 liter of deionized water to make 4X concentrate. Using pH meter, determine the pH of the solution. Solution should be between pH 9.0 and pH 9.5. Dilute to 1X. Direct FA using VMRD, Inc. FIP Direct FA Conjugate (catalog no. CJ-F-FIP-1ML or CJ-F-FIP-10ML) and FIP 2 well control slide (catalog no. SLD-FAC-FIP).

**Results:** Reconstituted Rinse Buffer was clear at the 4X solution as well as the 1X solution. The pH of the rinse buffer at 1X was 9.24. No difference was detected between the reference and QC rinse buffer on the FIP slides.

**INTENDED USE:** FA Rinse Buffer is used to rinse FA substrate slides.

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 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.