## Preparation of buffers and solutions for RABV ELISA

### Coating buffer: Carbonate-bicarbonate buffer, pH 9.6

* To a bottle containing approximately 800 mL of autoclaved deionized water, add 3 g of Na2CO3 and 6 g of NaHCO3
* Mix on a magnetic stir plate for 30 mins at RT
* Measure pH and adjust accordingly to pH 9.6 ± 0.2
* Top up the volume to 1 L, mix by inverting the bottle several times, then remeasure and record the pH
* Sterilize 250 mL of freshly prepared coating buffer by vacuum filtration (0.22 µM filter)

The preparation can be stored at +4 °C ± 3 °C for up to 3 months

### Dulbecco’s 1x PBS, without Ca++, Mg++

If using 10x concentrated PBS without Ca++ Mg++:

* + Dilute the concentrated Dulbecco’s PBS without Ca++/Mg++ 1:10 in H2O

The preparation can be stored at +5°C ± 3°C for up to 1 month

### Blocking buffer: 1:4 Block ACE

For 2 plates (adjust as necessary):

* + Reconstitute Block ACE by adding one 4 g sachet to 100 mL of sterile water
  + Mix by inverting several times
  + Add 20 mL of the solution to 60 mL of sterile water
  + Mix by inverting several times, and the blocking buffer is ready to use

Buffer is prepared for daily use

### Wash buffer: PBS/0.05%Tween20 (PBS-T)

Prepare the volume of Wash buffer (PBS-T) required for the washing of all plates (approximately 1 L for two plates).

* + Prepare 1x PBS by adding 100 mL 10x DPBS to 900 mL deionized water
  + Add 500 µL Tween20 to the solution
  + Mix by inverting several times

Washing volume should be adapted according to the available equipment:

• 500 µL/well if using an automated ELISA microplate washer (with liquid aspiration) or

• 300 µL/well if performing manual washing

Buffer is prepared for daily use.

### Diluent buffer: 1:10 Block ACE

A volume of 40 mL is sufficient for 2 plates

* + Add 4 mL of reconstituted Block ACE to 36 mL deionized water
  + Mix by inverting several times

Buffer is prepared for daily use

### Substrate solution: OPD (0.5 mg/mL)

Just before use (for two plates):

* Dissolve two tablets of O-phenylenediamine dihydrochloride (OPD) in 18 mL of deionized water, then add 2 mL of 10x stable peroxidase buffer and mix well before use

Use the preparation immediately after the preparation

### Stop solution H2SO4 (2N, 5%)

Corrosive solution: please strictly follow the dilution sequence:

* Prepare 800 mL H2O, then add 56.4 mL of 95%–97% H2SO4
* Bring to 1 L with H2O
* Stir the solution using a magnetic stirring bar

## Preparation of working concentrations of reagents

### Capture mAb (clone 1112-1; see Table 1)

Stock solution (reconstituted in sterile water):

* Gently spin the vial to collect the lyophilized content at the bottom of the vial
* Reconstitute the lyophilized capture mAb (clone 1112-1, 0.5 mg) according to the manufacturer’s instructions to obtain a solution at 0.5 mg/mL
* Gently spin the vial to collect the liquid at the bottom of the vial
* Keep this stock solution in 100 µL aliquots at -20°C

Working solution:

For 2 plates and a working concentration of 2.5 µg/mL:

* Dilute 50 µL of the stock solution of mAb clone 1112-1 in 10 mL of coating buffer to obtain a working solution with the final concentration of 2.5µg/mL (dilution factor from stock solution: 1:200)

Use immediately after preparation

### Detection mAb (clone D1-25, biotinylated; see Table 1)

Stock solution (reconstituted in sterile water):

* Gently spin the vial to collect the lyophilized content at the bottom of the vial
* Reconstitute the lyophilized capture mAb (clone D1-25, 0.5 mg) according to the manufacturer’s instructions to obtain a solution at 0.5 mg/mL
* Gently spin the vial to collect the liquid at the bottom of the vial
* Keep this stock solution in 50 µL aliquots at -20°C

Working solution:

For 2 plates and a working concentration of 0.2 μg/mL:

* Dilute 8.8 µL of the stock solution in 22 mL of diluent buffer to obtain a working solution with the final concentration of 0.2 µg/mL (dilution factor from stock solution: 1:2500)

Use immediately after preparation

### Detection conjugate: streptavidin-peroxidase ultrasensitive (see Table 1)

For 2 plates:

* Dilute 8.8 µL of the stock solution in 22 mL of diluent buffer to obtain a working solution with the final concentration of 0.4 µg/mL (dilution factor from stock solution: 1:2500)

Use immediately after preparation