

SERELISA[®] Rabies Ab Mono Indirect

KIT FOR THE DETECTION OF ANTI-RABIES ANTIBODIES IN DOG OR CAT INDIVIDUAL SERUM

INDIRECT IMMUNOENZYMATIC TECHNIQUE

192 SINGLE WELL REACTIONS

I. PRINCIPLE OF THE TEST

The Serelisa[®] Rabies Ab mono indirect kit allows a quantitative detection of Rabies antibodies in individual dog and cat serum samples. A minimum of 0.5 IU/ml Rabies antibodies is required to protect against Rabies infection, according to the World Health Organisation recommendations (WHO, 1992. Expert Committee on Rabies, 8th Report. World Health Organisation, Geneva, Technical Report Series n°824). The reaction is composed of three steps:

1. Each serum sample is placed in a well sensitised with inactivated Rabies viral antigens. Antibodies present in the sample bind to the viral antigens coated at the bottom of the well.
2. After a wash step, Protein A / peroxidase conjugate is added. It fixes to the immunoglobulins (antibodies) previously captured, forming a complex: (Rabies Ag) - (Ab anti-Rabies) - (Protein A/ peroxidase)
3. Excess conjugate is eliminated by a wash step. The enzyme linked to the complex is revealed by addition of a substrate which is transformed into a coloured product. After stopping the reaction, the optical densities are measured. The presence or absence of antibodies is determined by using threshold values obtained from the positive control.

II. KIT COMPOSITION AND CONSERVATION

REAGENT NATURE	RECONSTITUTION AND CONSERVATION
Microplate containing six 16-well strips sensitized with Rabies antigens	Use within 4 weeks after opening of the sachet which must be closed after use.
Conjugate : Protein A / peroxidase (CJ) (10X concentrated)	Dilute 10 times in the conjugate diluent and use within 24 hrs following dilution.
Buffered peroxidase substrate (PS)	Ready-to-use.
Negative control (N) (10X concentrated)	Dilute 10 times in the sample diluent and use within 24 hrs following dilution.
Positive control (P) (10X concentrated)	Dilute 10 times in the sample diluent and use within 24 hrs following dilution.
Sample diluent (SD)	Ready-to-use.
Wash solution (W) (10X concentrated)	Dilute 10 times in distilled or demineralized water. Use within 48 hrs following dilution.
Conjugate diluent (CD)	Ready-to-use.
Stop solution (S)	Ready-to-use.
Adhesive film	6 films

Note: Store diluted reagents at +5°C ± 3°C and use as mentioned above.

III. MATERIALS AND REAGENTS REQUIRED (NOT SUPPLIED)

- OIE reference serum
- Distilled or demineralized water.
- Adjustable or set pipettes to measure and deliver between 0 to 1000 µl. Measurement deviation must be ≤ 10% for volumes ≤10 µl and ≤ 5% for all other volumes.
- Graduated cylinders (100 ml and 1000 ml).
- Manual, automatic or semi-automatic washing device for microtitration plates.
- Microplate reader, fitted with filters for bichromatic reading at 450 and 630 nm. It is also possible to use a monochromatic reader fitted with a 450 nm filter.
- Incubator at +37°C ± 3°C.

IV. PRECAUTIONS FOR USE

The quality of the results depends on the respect of good laboratory practices and the procedure (see paragraph VI).

1. Do not mix or associate reagents from kits with different batch numbers
2. Do not use reagents after the expiry date.
3. Place all reagents at laboratory temperature for at least 1 hour prior to use.
4. Handle all reagents and samples as biohazardous material.
5. Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
6. Never pipette by mouth.
7. Avoid inter sample contamination during sample collection, storage or transport. Use separate disposable pipette tips for each sample.
8. Avoid contamination of the substrate solution with metallic ions, oxidizing agents or detergents. Make sure that all containers are clean. Do not use the same container or the same pipette tip for the conjugate and the substrate.
9. It is recommended to dispose reagents and contaminated material according to the applicable regulations. The safety data sheets for the product are available upon request.

Risk phrases :

- R 23/25 : Toxic by inhalation and if swallowed.
R35 : Causes severe burns.
R 36/37/38 : Irritating to eyes, respiratory system and skin.
R 41 : Risk of serious damage to eyes.
R 42/43 : May cause sensitization by inhalation and skin contact.
S 7 : Keep container tightly closed.
S 24 : Avoid contact with skin.
S26 : In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S30 : Never add water to the product.
S45 : In case of accident or if you feel unwell, seek medical advice immediately.

V. SAMPLES

The reaction is performed on heat-inactivated (30 min. +56°C) individual serum diluted at 1:100. Testing the appropriate set of dilutions for the OIE Rabies standard serum containing 6.7 IU/ml is necessary. This standard serum is supplied by the OIE Reference Laboratory for Rabies, Nancy, France. Serum samples should be stored as follows:

Samples	Cold (+5°C)	Freeze (-20°C)	Lab Temperature (20°C)
Serum	max. 7 days	Yes	No

VI. PROCEDURE

Strictly comply with the procedure indicated below. Use negative and positive controls in duplicate for each test run, or at least for each plate.

A. PRELIMINARY STEPS.

- 1 - Carefully set up the distribution and identification of controls and samples.
- 2 - Prepare the sera to be tested. Dilutions are performed in the kit sample diluent (SD). Dilutions should be performed as follows:

the samples are first pre-diluted at 1:10 in a blank microplate (10 µl of sample in 90 µl of SD)

3 - For serum titration, a set of seven dilutions of the OIE Rabies standard serum should be performed either in tubes or in microplates with an initial dilution of 1:10, 1:30, 1:100, 1:150, 1:300, 1:1000 and 1:3000.

We recommend the following scheme to prepare the appropriate set of dilutions:

OIE dilution	Preparation
1:10	10 µl of OIE + 90 µl of sample diluent SD
1:30	10 µl of OIE + 290 µl of SD
1:100	10 µl of 1/10 dil + 90 µl of SD
1:150	20 µl of 1/30 dil + 80 µl of SD
1:300	10 µl of 1/30 dil + 90 µl of SD
1:1000	10 µl of 1/100 dil + 90 µl of SD
1:3000	10 µl of 1/300 dil + 90 µl of SD

This range of dilution of OIE standard serum shall be present in every serial and plates.

B. TEST PROCEDURE.

I - CONTROLS AND SAMPLES DISTRIBUTION

1. Control distribution:

Controls are not ready-to-use and should be diluted at 1:10.

Dispense 90 µl of sample diluent, and add 10 µl of the kit negative control into wells A1 and A2, and 10 µl of the positive control to wells B1 and B2.

2. Distribution of samples and OIE serum dilutions:

Dispense 90 µl of sample diluent, add 10 µl of either 1:10 sample pre-dilution or each OIE serum dilution from 1:10 to 1:3000 into the test wells and mix gently.

- Strips should always be placed on the frame so that both washer and reader can be used.

- Cover the wells with adhesive film, cut to the necessary length by the number of strips used.

- Mix the plate manually by gentle shaking or by using a plate agitator.

	1	2	3	4
A	N 1:10	N 1:10	OIE 1:30000	OIE 1:30000
B	P 1:10	P 1:10	S1 1:100	S1 1:100
C	OIE 1:100	OIE 1:100	S2 1:100	S2 1:100
D	OIE 1:300	OIE 1:300	S3 1:100	S3 1:100
E	OIE 1:1000	OIE 1:1000	S4 1:100	S4 1:100
F	OIE 1:1500	OIE 1:1500	S5 1:100	S5 1:100
G	OIE 1:3000	OIE 1:3000	S6 1:100	S6 1:100
H	OIE 1:10000	OIE 1:10000	S7 1:100	S7 1:100

Antibody quantification (final dilution)

Over OD values may be observed for the 1:100 OIE dilution. In this case, use the 6 following OIE dilutions to perform the regression curve.

3. Incubation of the plate

1 hour ± 5 min. at +37°C ± 3°C.

WASHING:

Wash buffer: dilute the concentrated washing solution (W) 1:10 in distilled or demineralized water.

Carefully remove the adhesive film and wash 4 times.

II – ADDITION OF THE CONJUGATE

1. Preparation of the conjugate:

Dilute the concentrate (CJ) 1:10 in the conjugate diluent (CD). 2 ml are needed for one strip, meaning 200 µl of CJ in 1.8 ml of CD.

2. Distribution of the conjugate:

Add 100 µl of diluted conjugate to all the wells and cover with a new piece of adhesive film.

3. Incubation of conjugate:

1 hour ± 5 min. at +37°C ± 3°C.

WASHING:

Carefully remove the adhesive film and wash 4 times.

III – REVELATION

1. Addition of the substrate:

Add 100 µl of buffered peroxidase substrate (PS) per well. Do not cover with adhesive film at this stage. Mix by gentle shaking the plate manually or use a plate agitator to ensure correct homogenisation.

2. Incubation of substrate:

Incubate for 30 minutes ± 5 min. at laboratory temperature (+20°C ± 5°C), shielded from light.

3. Addition of the Stop Solution:

Add 50 µl of stop solution (S) per well.

Mix by gentle shaking the plate manually or by using a plate agitator. Make sure that no bubbles occur in the wells.

4. Measure of the optical density:

Measure the optical density (OD) bichromatically at 450 and 630 nm or monochromatically at 450 nm (in the yellow band).

Reading bichromatically is strongly recommended. Should a monochromatic reader be used, ensure the cleanliness of the bottom of the wells prior to reading.

VII. TEST VALIDATION

The results of each test run (or for each plate) are valid :

- when the optical density (OD) obtained with the positive control is ≥ 0.300 , and

- when the optical density (OD) obtained with the negative control is $< 0.50 \times OD P$, and

- when the correlation coefficient between the Neperian logarithm (ln) ODs and ln Rabies Ab concentrations for the OIE standard serum is > 0.95 .

VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

Method: TITER CALCULATION USING THE REGRESSION CURVE

(We recommend the use of an Excel spreadsheet. Synbiotics will provide you with a ready-to-use file upon request)

- Calculate the average OD value for each sample tested and each OIE serum dilution.

- Calculate the Neperian logarithm (ln) value for each average OD and the ln value of the Rabies Ab concentration for each OIE dilution (from 6.7 to 0.0223 IU/ml, without taking into account the 1:100 testing dilution factor).

- Plot the ln (OD) (Y-axis) as a function of the ln (Rabies Ab concentration) (X-axis) in order to draw the reference curve for the OIE standard serum.

- Using all individual results obtained for the OIE standard serum dilutions, perform a linear regression curve between ln Rabies Ab concentrations (expressed in EU/ml) (equivalent units per ml) and ln (OD), to establish the corresponding mathematics model :

$$\ln [\text{Rabies Ab concentration (EU/ml)}] = a + b \cdot \ln OD$$

- For each tested sample, calculate the average OD value and then the Rabies antibody concentration of the sample expressed as «equivalent units per ml» (EU/ml), from the established model:

$$\text{Sample Rabies Ab concentration (EU/ml)} = e^{(a + b \cdot \ln OD)}$$

- If the calculated titre > 0.6 , the animal is considered as protected.

- If the calculated titre is < 0.6 , the animal is considered as not protected (a confirmation using FAVN may be performed).

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EXAMPLE

* Positive control :

$$\text{well B1} = 0.610; \text{well B2} = 0.690 \rightarrow \overline{\text{OD P}} = 0.650$$

* Negative control :

$$\text{well A1} = 0.190; \text{well A2} = 0.210 \rightarrow \overline{\text{OD N}} = 0.200$$

$$\text{* Sample 1 : } \text{OD 1} = 1.790 \quad \text{OD 2} = 1.750 \rightarrow \overline{\text{OD}} = 1.770$$

$$\text{* Sample 2 : } \text{OD 1} = 0.350 \quad \text{OD 2} = 0.390 \rightarrow \overline{\text{OD}} = 0.370$$

* Test validation : $\overline{\text{OD P}} = 0.650 > 0.300$ et $\overline{\text{OD N}} = 0.200 < 0.50 \times 0.650 = 0.325$, therefore valid test.

Samples (final dilution)	Ab UI/ml	OD 1	OD 2	average OD	ln [conc. Ab]	ln (average OD)
OIE 1:100	6.7	over	over	over	1.9021	-
OIE 1:300	2.233	1.280	1.237	1.259	0.8033	0.2299
OIE 1:1000	0.67	0.809	0.751	0.780	-0.4005	-0.2485
OIE 1:1500	0.447	0.600	0.620	0.610	-0.8052	-0.4943
OIE 1:3000	0.2233	0.406	0.425	0.416	-1.4992	-0.8783
OIE 1:10000	0.067	0.214	0.217	0.216	-2.7031	-1.5348
OIE 1:30000	0.0223	0.148	0.154	0.151	-3.8032	-1.8905
Sample 1 1/100	unknown	1.790	1.750	1.770	unknown	0,5710
Sample 2 1/100	unknown	0.350	0.390	0.370	unknown	-0,9943

Mathematics Model :

$$\ln [\text{Rabies Ab}] = 0.255 + 2.063 * \ln \text{OD}$$

* Test validation : correlation coefficient $r = 0.996 > 0.95$, therefore valid test

* Rabies Ab concentration in Sample 1 :

$$e^{(0.255 + 2.063 * \ln \text{OD})} = e^{(0.255 + 2.063 * 0.5710)} = 4.19 \text{ EU /ml} \rightarrow \text{protected}$$

* Ab Concentration in sample 2 :

$$e^{(0.255 + 2.063 * \ln \text{OD})} = e^{(0.255 + 2.063 * -0.9943)} = 0.17 \text{ EU /ml} \rightarrow \text{not protected (FAVN confirmation)}.$$