# BioPro Rabies ELISA Ab Kit

## Blocking ELISA for detection of Rabies virus antibodies in serum or plasma

### 480 reactions

**Cat. No.: RAB01-05**
**Version: Rabies 1.1c**

**Instruction for use.**

**Manufacturer**

**O.K. SERVIS BioPro, s.r.o.**

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## INTRODUCTION

Rabies is fatal viral disease occurring in humans and animals. Disease is caused by highly neurotropic Rabies virus. Virus is mostly transmitted through close contact with infected saliva of rabid animals via bites or scratches and then through neural path to the CNS causing encephalopathy and ultimately death.
Domesticated and wild carnivores belong to most infected animals. For eradication of Rabies in wild carnivores oral immunisation in many country is used. Estimation of seroprevalence in vaccinated population is one of the methods for evaluation of oral immunisation effectiveness.

ELISA Rabies kit was developed for detection of antibodies against Rabies in domesticated and wild carnivores and was validated with fox and dog serum samples. We recommend use this ELISA for detection of antibodies against Rabies in foxes. This method is rapid and simple and in comparison with “golden standard” methods like FAVN or RFFIT is more convenient because very often quality of fox serum samples may be decreased by bacterial contamination or autolysis.

## PRINCIPLE OF TEST

The wells of microplates are coated with Rabies antigen. Diluted samples are incubated in the wells. After washing biotinylated anti-Rabies antibody is added to wells. If investigated sample contain specific anti-Rabies antibodies, these block binding of biotinylated anti-Rabies antibodies with coated Rabies antigen. If investigated sample do not contain specific anti-Rabies antibodies, biotinylated anti-Rabies antibodies form antigen-biotinylated antibody complex. After washing Streptavidin peroxidase conjugate is added to wells. Streptavidin peroxidase will bind to antigen-biotinylated antibody complex. After another washing step substrate solution (TMB) is added to wells, forming blue compound becoming yellow after stopping reaction. Intensity of colour is read at 450 nm and decrease of the intensity compared against negative control is proportional to amount of blocking antibodies in investigated sample.

## REAGENTS

The kit should be stored on a dry and dark place at 2 - 8°C.

| | |
|---|---|
| ▪ Rabies coated microplates | 5 x 96 well strip plates |
| ▪ Rabies positive control serum | 2 vials (0,6 ml), red cap |
| ▪ Rabies negative control serum | 2 vials (0,6 ml), blue cap |
| ▪ Rabies control serum 1 | 2 vials (0,3 ml), black cap |
| ▪ Rabies control serum 2 | 2 vials (0,3 ml), white cap |
| ▪ Rabies control serum 3 | 2 vials (0,3 ml), transp. |
| ▪ Sample diluent (ready to use) | 2 bottles (15 ml) |
| ▪ Diluent for biotinylated antibody (ready to use) | 2 bottles (30 ml) |
| ▪ Biotinylated anti-Rabies antibody (100x concentrated) | 2 vials (0,3 ml), yellow |
| ▪ Diluent for Streptavidin peroxidase conjugate (ready to use) | 2 bottles (30 ml) |
| ▪ Streptavidin peroxidase conjugate (100x concentrated) | 2 vials (0,3 ml), green cap |
| ▪ TMB Substrate (ready to use) | 2 bottles (30 ml) |
| ▪ Stop solution; 0,5M $H_2SO_4$ (ready to use) | 2 bottles (15 ml) |
| ▪ Washing solution (10x concentrated) | 2 bottles (250 ml) |
| ▪ Adhesive foils | 15 foils |

## MATERIAL REQUIRED BUT NOT INCLUDED IN THE KIT

Precision Micropipettes and Multichanel micropipette for volume from 10 to 1000 μl

Disposable pipette tips 10-1000 μl

Manual or automatic microplate washing system

Microplate reader, filter 450 nm

Vortex, orbital shaker

Incubator (37 °C)

Graduated flask (50-1000 ml)

Centrifuge tubes

Distilled or deionised water

## PRECAUTIONS FOR USE

- Store all reagents at 2 - 8°C.
- All the reagents of the kit must be allowed to come to the room temperature (18 - 25°C) before use, except biotinylated antibody and streptavidin peroxidase conjugate.
- Consider any material directly in contact with samples and the reagents as potentially infectious material.
- Avoid contact of TMB substrate with skin, mucous membranes and eyes.
- Wear disposable gloves when handling samples and reagents.
- Do not pipette with mouth.

- Use new disposable tips for every investigated sample.
- Do not use reagents after expiration date and do not mix reagents from different lots.
- Stop solution contains acid (0,5M $H_2SO_4$), that can cause serious burns. In case of contact with skin and eyes, wash immediately with plenty of water and seek medical advice.
- Distilled or deionised water of good quality should be used for preparation of washing solution.
- Avoid foam and bubble formation by careful pipetting and washing.
- During the test procedure plate must never become dry. If the test procedure is discontinued for any reasons leave the wells of the plate filled with wash solution.
- Protect TMB Substrate from direct light and oxidising agents. Use only clean laboratory glass or plastic with TMB Substrate. Do not use TMB substrate showing blue colour before dispensing to the wells.
- Before discarding all used materials and disposables should be decontaminated either by immersion for at least 1 hour in freshly prepared 5% sodium hypochloride, or by autoclaving at 121°C for 30±1 minutes minimum.

## PREPARATION OF REAGENTS

### Preparation of microplates

Microplates should be allowed to come to room temperature (18 - 25°C) before use. Opened unused strips may be stored in closed plastic bag with desiccant bag at 2 - 8°C in the dark no more than one month.

### Wash solution

Concentrated wash solution (10x) should be brought to room temperature before use. May form crystals at 2 - 8°C, which will disappear at the room temperature (18 - 25°C). If they do not disappear, solution should be left at the room temperature for longer time or it is possible to warm solution to 37±2 °C and gently shake till all the crystals will be dissolved.

To prepare wash solution mix 1 volume of concentrated wash solution (10x) with 9 volumes of distilled or deionised water (e.g. 50 ml of concentrated wash solution (10x) add to 450 ml of distilled or deionised water).

### Biotinylated anti-Rabies antibody

Dilute concentrated biotinylated anti-Rabies antibody to 1/100 in Diluent for biotinylated antibody. (e.g. quantity needed for one plate is represented with mix of 110 µl concentrated biotinylated anti-Rabies antibody and 11 ml Diluent for biotinylated antibody).

Before preparation of working dilution, concentrated biotinylated anti-Rabies antibody must to be vortexed.

Prepared working dilution of antibody has to be used within 8 hours!

### Streptavidin peroxidase conjugate

Dilute concentrated Streptavidin peroxidase conjugate to 1/100 in Diluent for Streptavidin peroxidase conjugate (e.g. quantity needed for one plate is represented with mix of 110 µl concentrated biotinylated Streptavidin peroxidase conjugate and 11 ml Diluent for Streptavidin peroxidase conjugate).

Before preparation of working dilution, concentrated Streptavidin peroxidase conjugate must to be vortexed.

Prepared working dilution of Streptavidin peroxidase conjugate has to be used within 8 hours!

## PREPARATION OF SAMPLES

Investigated serum samples need to be diluted to 1/2 in Sample diluent (e.g. 60μl + 60μl) in a dummy plate or in microtubes. Positive control serum, negative control serum and control sera needs to be diluted in the same manner as serum samples.

Note:

1. As a dummy plate any low protein binding microplate can be used.
2. It is also possible dilute controls and serum samples directly in plate. Dispense 50μl of Sample diluent per well and then dispense 50 μl of positive control serum, negative control serum and control sera into appropriated wells. Then dispense 50μl of serum samples to the remaining wells.

## TEST PROCEDURE

### 1. Incubation of investigated and control sera

Note: All the control sera have to be vortexed before dispensing.

1.1 Dispense 100μl of diluted positive control serum to A1 and B1 wells.
1.2 Dispense 100μl of diluted negative control serum to A2 and B2 wells.
1.3 Dispense 100μl of diluted control serum 1 to well C1.
1.4 Dispense 100μl of diluted control serum 2 to well D1.
1.5 Dispense 100μl of diluted control serum 3 to well E1.
1.6 Dispense 100μl of diluted serum samples to the remaining wells.
1.7 Cover the plate with adhesive foil and incubate overnight (18 - 24 hours) at 2 - 8°C with gently shaking on orbital shaker.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | PC | NC | 10 | | | | | | | | | |
| B | PC | NC | 11 | | | | | | | | | |
| C | CS1 | 4 | 12 | | | | | | | | | |
| D | CS2 | 5 | 13 | | | | | | | | | |
| E | CS3 | 6 | … | | | | | | | | | |
| F | 1 | 7 | | | | | | | | | | |
| G | 2 | 8 | | | | | | | | | | |
| H | 3 | 9 | | | | | | | | | | |

**2. Incubation with biotinylated anti-Rabies antibody**

2.1 Remove foil from the plate, empty the content of the plate and wash 6 times with washing solution. Tap the plate firmly on the absorbent paper after last washing step.
2.2 Dispense diluted biotinylated anti-Rabies antibody 100µl per well.
2.3 Cover the plate with adhesive foil and incubate 30±1 minutes at 37± 2°C with gently shaking on orbital shaker.

**3. Incubation with Streptavidin peroxidase conjugate**

3.1 Remove foil from the plate, empty the content of the plate and wash 4 times with washing solution. Tap the plate firmly on the absorbent paper after last washing step.
3.2 Dispense diluted Streptavidin peroxidase conjugate 100µl per well.
3.3 Cover the plate with adhesive foil and incubate 30 minutes at 37± 2°C with gently shaking on orbital shaker.

**4. Incubation with TMB Substrate**

4.1 Remove foil from the plate, empty the content of the plate and wash 4 times with washing solution. Tap the plate firmly on the absorbent paper after last washing step.
4.2 Dispense 100µl of "ready to use" TMB Substrate per well.
4.3 Incubate the plate 15-30 minutes at room temperature (18 - 25°C) with gently shaking on orbital shaker, away from direct sunlight.

Note: In the case that shaking with orbital shaker during incubations is not available and all incubations were done without shaking, we propose to incubate the plate with TMB Substrate longer than 20 minutes.

**5. Stopping the reaction**

5.1 Dispense 50µl of Stop Solution per well.

**6. Reading**

6.1 Read optical density (OD) at 450nm.

## VALIDATION CRITERIA

- The OD of the negative control serum must be higher than 1,0.
- Difference between means of OD of negative and positive control serum must be equal or higher than 0,8.

If validation criteria are not met, test results of that specific test plate are invalid and samples have to be retested.

## PANEL OF CONTROL SERA:

Should be used as a help for user to assure that test is working in optimal conditions. If the criteria mentioned bellow are not achievable, please contact producer.
Percentage of blocking for control serum 1 should be between 45% and 70%.
Percentage of blocking for control serum 2 should be between 25% and 45%.
Percentage of blocking for control serum 3 should be lover than 30%.

## INTERPRETATION:

Calculate percentage of blocking (PB) for each sample:

$$PB\% = \frac{OD_{NC} - OD_{sample}}{OD_{NC} - OD_{PC}} \times 100$$

- Serum sample with **PB lower than 40**% is considered as negative for Rabies antibodies.
- Serum sample with **PB equal or higher than 40%** is considered as positive for Rabies antibodies.
- Serum sample with **PB equal or higher than 70%** is considered as serum sample with antibody level equal or higher than 0,5 IU/ml based on FAVN test.

Note: For the purpose of evaluation of oral immunisation effectiveness we recommend to use first positive cut off (PB equal or higher than 40%). To use second positive cut off (PB equal or higher than 70%) for interpretation of results with fox sera is not recommended due to the most fox serum samples are in fact body fluids with unknown dilution factor, which make almost impossible to quantify the exact level of protection.

## SCHEME OF TEST PROCEDURE

1. Dilute control sera and serum samples.
1.1 Dispense 100µl of diluted positive control serum to A1 and B1 wells.
1.2 Dispense 100µl of diluted negative control serum to A2 and B2 wells.
1.3 Dispense 100µl of diluted panel of control serum to wells A3-A5.
1.4 Dispense 100µl of diluted serum samples to remaining wells.
1.5 Incubate overnight at 2 - 8 °C, shake gently.
2. Prepare biotinylated anti-Rabies antibody.
2.1 Wash plates 6x time.
2.2 Dispense 100µl of biotinylated anti-Rabies antibody diluted to 1/100 to all wells.
2.3 Incubate 30 minutes at 37± 2°C, shake gently.
3. Prepare streptavidin peroxidase conjugate.
3.1 Wash plates 4x time.
3.2 Dispense 100µl of streptavidin peroxidase conjugate diluted to 1/100 to all wells.
3.3 Incubate 30 minutes at 37± 2°C, shake gently.
4.1 Wash plates 4x time.
4.2 Dispense 100 µl of TMB Substrate to all wells.
4.3 Incubate 15-30 minutes at room temperature (18 - 25°C), shake gently.
5.1 Stop reaction with 50 µl of Stop solution.
6.1 Read absorbance (OD) at 450 nm.
7.1 Validation criteria.
7.2 Interpretation of result.

**APPENDIX 1 to BioPro Rabies ELISA Ab kit – Instruction for use,v 1.1**
Date: 10.12.2013

Based on user inputs we have decided to add following information to Instruction for use of our BioPro Rabies ELISA Ab kit:

1. INSUFICIENT SAMPLE VOLUME

If there is not enough sera (less than 50µl) dilute such serum sample with PBS to achieve sample volume of 50µl (for example if you have 30 µl of serum add 20 µl of PBS). Mark such sample as diluted and follow standard test procedure, step 1.

2. OBTAINING SAMPLE FROM A BLOOD CLOT

Alternatively the sample can be obtained from a blood clot using following procedure:

2.1. FRESH BLOOD CLOT

- Cut the **fresh blood clot** (obtained from heart or major blood vessels) and transfer to 10-12 ml tube.
- Close the tube and leave at room temperature (18  25°C) or in incubator heated to 37±2°C for 2-3 hours.
- Transfer to refrigerator (4-8°C) for 12 hours ±30 minutes (overnight).
- Centrifuge the liquid part from blood clot at 5-10.000 rpm for 15±10mins and then test obtained liquid as serum following standard procedure, step 1.

2.2. DRIED BLOOD CLOT

- Cut the **dried blood clot**, put into 10-12ml tube, add PBS with antibiotics (PNC G 100000 U/l, STM 100mg/l, NEO 50 mg/l) maximally in 1:1 ratio, mark as diluted sample and follow according to point 2.1. of this appendix.

## Contact

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