

VacciGel™ Rabies Virus ELISA KIT #VAC-RBV-50, 50 tests

Instruction Manual No. M-VAC-RBV-50

## VacciGel™ Direct ELISA for the measurement of Rabies Virus Vaccine formulated in Alum

Cat. #. VAC-RBV-50; 50 Tests



For In Vitro Research Use Only



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Kit Components, 96 tests	Cat #
VacciGel™ Rabies Virus Std. <b>A</b> (0 U), 0.65 ml	VACRBV-51A
VacciGel™ Rabies Virus Std. <b>B</b> (3 U), 0.65 ml	VACRBV-51C
VacciGel™ Rabies Virus Std. <b>C</b> (11U), 0.65 ml	VACRBV-51D
VacciGel™ Rabies Virus Std. <b>D</b> (33 U), 0.65 ml	VACRBV-51E
VacciGel™ Rabies Virus Std. <b>E</b> (100 U), 0.65 ml	VACRBV-51F
<b>Note:</b> All Stds are made in Alum suspension. Shake well and mix before use.	
VacciGel™ Sample Diluent, pink solution, 15 ml ( <b>mix the contents prior to use</b> )	VACRBV-52
Anti-RBV-HRP Conjugate, 90 ul ( <b>100X</b> ), Dilute 1:100 with 1X Sample/Antibody Conjugate. Diluent	VACRBV-53
Sample/Antibody Conjugate Diluent, 10 ml ( <b>20X</b> ) diluent 1:20 with water	SD-20T
Low NSB Diluent (Green solution), 15 ml ( <b>mix the contents prior to use</b> )	TBTm
Wash buffer (100X), 10 ml; dilute 1:100 with distilled water	WB-100
HRP substrate, Solution, 12 ml	80091
Stop solution, 12 ml	80101
ELISA Strip Plate (8x12 or 96 wells)	VAC-P1
VacciGel™ Assay Tubes, 50	VACT-50
VacciGel™ Qtips, 50	VACQ-50
Instruction Manual	VAC-HBS-50

A vaccine is a biological preparation that improves immunity to a particular disease. Some vaccines also contain chemicals called adjuvants to help stimulate the production of immunity against the vaccine active ingredients, making the vaccine more effective. Currently, the only adjuvants approved for human vaccine are aluminum containing compounds, including aluminum hydroxide, aluminum phosphate, and potassium aluminum sulfate or alum. Aluminium adjuvants have been used in tetanus, diphtheria, pertussis, polio, rabies, and hepatitis A and B vaccines.

To ensure vaccine quality, regulatory authorities require the manufacturer to measure vaccine content in the final product. World Health Organization (WHO) recommends that at least 80% of the vaccine be adsorbed to the gel. In particular, it is essential to determine the amount as well as the identity and integrity of the antigens bound to aluminum containing adjuvants following formulation. Aluminum-based gels are typically fibrous or beaded in suspension. The presence of aggregates, turbidity, flocculent gels or beads in solution prevents direct quantitation of protein content in formulations using protein assays such as Lowry, BCA, or Bradford protein assay, not to mention that these assays are all non-specific and low in sensitivity. Alum formulations also do not allow complete dissolution or extraction making it very difficult to know the identity of the vaccines or know the amount of the protein after their dispensing. There have been several incidents of mislabeling of anti-fertility vaccine with tetanus vaccines. Therefore, there is an urgent need for a test not only to identify but measure the vaccine contents.

The VacciGel™ ELISA for Rabies Virus is the first commercial test to measure the active component of the vaccine in vaccines formulated in Aluminum hydroxide or Alum gel. It is a simple, rapid, and sensitive test and required no extraction or harsh dissociation of the antigens from the gel (Alum). This kit has been validated with IMRAB® (Merial) rabies vaccine for animals which contains the same virus strain used in the human vaccine.

## PRINCIPLE OF THE TEST

VacciGel™ Rabies Virus ELISA kit is based on direct binding of anti-Rabies virus antibody-HRP conjugate to Rabies virus adsorbed on the gel. After a washing step, chromogenic substrate is added and color is developed. The enzymatic reaction (color) is directly proportional to the amount of Rabies Virus present in the sample. Adding a stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of Rabies virus in the samples and controls are read off the standard curve.

## MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipette (5-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader. Table top microfuge

## PRECAUTIONS AND SAFETY INSTRUCTIONS

ADI VacciGel™ ELISA kit is intended for *in vitro research* use only. The reagents contain proclin-300 (0.1%) as preservative; necessary care should be taken when disposing solutions.

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). <http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf>

## SAMPLE COLLECTION AND HANDLING

This kit is designed to measure the Rabies virus in vaccines formulated in Alum. Do not add azide or other preservatives to vaccines. Do not freeze the vaccines. This kit is not suitable to measure Rabies in serum or plasma or Rabies in solution (without Alum). ADI has other kits to measure HBsAg in serum or plasma or biological buffers.

## REAGENTS PREPARATION FOR THE ASSAY

**Dilute wash buffer (1:100)** with distilled water (10 ml WB-100 in 990 ml). Store at 2-8°C.

**Prepare 1X Sample Diluent:** Dilute SD-20T 1:20 with water. Prepare as much necessary for the day. Return the stock 2-8°C

**Samples Dilution:** We suggest starting dilutions of 1:5 to 1:150 for testing. We suggest to dilute the vaccine in 2-steps: 1) Make an initial 1:5 stock of vaccine samples (e.g., 10 ul of the vaccine and 40 ul of Pink VacciGel™ diluent). This stock can be used to prepare further test dilutions of 1:10 or higher in Vaccine diluent (pink solution; 50 ul of 1:5 stock into 50-ul of vaccine diluent for a final dilution of 1:10). Unused Rabies vaccine samples should be stored in the 1:5 stock solution as the diluent has protein additives and preservatives. Diluted vaccines are stable in this diluent for up to 4-6 weeks. Dilution of all samples must be done in the supplied VacciGel™ diluent (pink solution) only. Do not use any other diluent. In case of unknown concentrations of the sample, prepare several dilutions of the vaccine in the VacciGel™ diluent. We recommend all samples be tested in duplicate.

**Dilute enzyme conjugate 1:100** (Ex. 10 ul of HRP-conjugate in 1X 990 ul SD-20T). Prepare only in required amounts for the days assay. Do not keep 1X HRP solution beyond the assay date.

## STORAGE AND STABILITY

The kit contents, if unopened, are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of shipping under appropriate storage conditions..


**TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).** **Important: If you have not used this kit before, we recommend to run the standards (0, 3U, 11U, 30U, and 100U) to get familiar with the test and not run the risk of making mistakes and losing samples or the whole kit.**

We recommend all standards, controls, and samples be tested in duplicate. Remove the required number of supplied 1.5 ml assay tubes, corresponding the number of tests. Store unused tubes and strips in the supplied bag. **Dilute wash buffer 1:100** with distilled water. **Dilute HRP conjugate 1:100** in 1X SD-20T. IMRAB® (Merial) is supplied in Alum. It is a gel suspension. Gently mix the vaccine gel suspension by inverting the contents a few times and then gently mixing it for 5-10 mins at room temperature. The vaccine suspension, if not mixed will settle at the bottom. Therefore, take the samples for testing immediately after mixing. Dilute vaccine samples in the supplied VacciGel™ diluent only (Pink solution).

1. Label the required number of 1.5 ml assay tubes and collect the required number of blank ELISA strips as well. Do not waste the assay tubes or the blank strip wells. If necessary break off the wells and arrange them on the well holder. The ELISA wells are only used to read the A450 values of the samples at the end of the assay. It is possible to use ELISA strips or ELISA plates from other suppliers as well.
2. Dilute Vaccine samples formulated in Alum **1:5-1:100** (or as necessary) in VacciGel™ diluent only (pink solution). IMRAB® Rabies vaccine is supplied at a proprietary concentration. Our own testing showed a 1:5 to 1:200 dilution to be within the range of the assay. Note: The gel will settle at the bottom during storage. It should be gently mixed for 5-10 seconds before use. .
3. VacciGel™ stabilizer solution (green) is a bit turbid or may have a cloudy appearance. It should be gently mix by manual shaking or inverting the bottle for 5-10 seconds prior to every use. **Pipet 300 ul of stabilizer solution** to an appropriate number of labeled 1.5 ml conical assay tubes supplied in the kit.
4. **Do not dilute the standards.** Gently mix the standards by vortexing for 5-10 seconds. **Dispense 100 ul of the standards, vaccine samples** (diluted appropriately) in duplicate into the tubes containing **300-ul green diluent. Close the caps and gently mix the contents by vortexing for 2-3 seconds; incubate for 30 mins at room temperature.**
5. Centrifuge the tubes for 60 seconds in a microfuge at 3,000 rpm at room temperature. Time and speed may have to be adjusted based on each centrifuge. **Note:** The pinkish/brownish small pellet of Alum gel at the bottom of all tubes. Carefully invert the tube and discard the supernatant in a waste container. Keep the tubes inverted and tap over a paper towel a few times to remove any remaining solution.

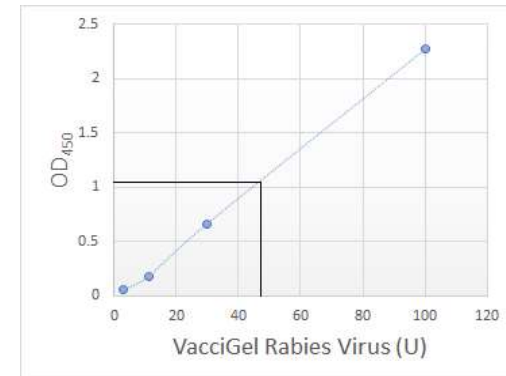
You must not disturb the gel pellet or discard it as it contains the vaccine active ingredients. The pellet will remain at the bottom of the tube during the process. Return all tubes to the tube holder.

## WORKSHEET OF TYPICAL ASSAY

- Add **150 ul of the working dilution (1:100) of antibody-HRP conjugate**. Vortex each tube 3-5 seconds to mix the pellet with the conjugate solution. **Note:** The gel pellet must have a uniform suspension, failure to achieve a uniform suspension will lead to lower OD<sub>450</sub> or high variance in duplicates. After mixing, **incubate all tubes for 60 minutes** at room temperature.
- Centrifuge the tubes for 60 secs in a microfuge at 3,000 rpm at room temperature as in step 5. **Note** the small pellet of Alum gel at the bottom of each tube. Carefully invert the tube and remove the conjugate solution as in step 5. Wash the pellet by adding **300 ul** of 1x wash buffer into all tubes. Vortex to mix and resuspend the pellet to make uniform suspension. Repeat the pellet wash 4 times more for a total of 5-washes. **Note:** After each wash, the tubes must be tapped over the paper towels to remove the liquid. Failure to wash properly will produce higher non-specific binding.
- After the last wash, remove all liquid from the tube or the walls and tap over fresh paper towels. Observe each tube for any liquid or droplets sticking on the tube walls. Remove traces of HRP-conjugate from the tube walls using supplied Q-tips. Failure to remove the wash solution will result into higher blanks. Do not disturb the pellet or touch with the Qtip.
 
- Pipette 150 ul of TMB substrate solution per tube**. Close the cap and vortex each tube for 3-5 seconds to make sure that the gel pellet is completely dispersed. Failure to perform this step properly will give spurious readings or irregular duplicates. **Incubate for 15 minutes at room temperature**. Incubation time may be changed  $\pm$  5 minutes to obtain a high standard of OD<sub>450</sub> 2.00-3.00 if necessary. Blue color should develop in standards and positive samples.
- Stop the reaction by adding 150 ul of stop solution** to all tubes. Mix gently for 3-5 seconds to ensure even color distribution. Blue color turns yellow.
- Centrifuge the tubes for 60-seconds at 3,000 rpm. Carefully take 200 ul of the supernatant (yellow) using a pipette and transfer to the ELISA strip wells for reading**. The order of the wells should be the same as for the tubes.
- Measure the absorbance at 450 nm using an ELISA reader. Color is stable for at least 30 minutes after stopping.

Wells	Stds/samples	Mean A <sub>450</sub> nm	Net A <sub>450</sub> nm
A1, A2	VacciGel™ Std. A (Blank or 0)	0.069	0
B1, B2	VacciGel™ Std. B (3 U)	0.123	0.054
C1, C2	VacciGel™ Std. C (11 U)	0.244	0.175
D1, D2	VacciGel™ Std. D (30 U)	0.733	0.664
E1, E2	VacciGel™ Std. E (100 U)	2.338	2.269
F1, F2	Sample 1	1.06	0.991

NOTE: This data is for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Blank should not be used in calculation of the curve, only to normalize standard and sample values. Each laboratory should determine their own normal reference values.



A typical assay standard curve (do not use this for calculating sample values)

### CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on a graph paper by plotting net absorbance values of standards against appropriate Rabies virus concentrations. Read off the Rabies concentrations of the control and samples directly from the standard curve. If samples were diluted then the values should be multiplied by the dilution factor.

If an ELISA reader software is being used, we recommend 4-parameter or 5-parameter curve.

### VacciGel™ Standard Calibration

Rabies vaccine Standards and controls are calibrated with USDA-approved IMRAB® Merial vaccine formulated in Alum. Vaccines from other manufacturers may give a different concentration due to the Rabies inactivation protocol, virus strain, or the formulations of Alum or % of Aluminum. Therefore, we recommend that an internal reference is used

### Sample Dilution and recovery

Rabies Vaccine samples (IMRAB®) when diluted 1:10, 1:20, 1:40 in VacciGel™ diluent (pink solution) showed good recoveries.

## PERFORMANCE CHARACTERISTICS

**Detection limit-** Based on replicate determinations of the zero standard, the minimum Rabies vaccine concentration detectable using this assay is ~1:200 dilution of the vaccine. The detection limit is defined as the value deviating by 2 SD from the zero standard.

### Specificity

VacciGel™ Direct Rabies Virus ELISA has been tested and calibrated with USDA-approved Rabies vaccine IMRAB® by Merial (Animal formulation). IMRAB® Vaccine is a Rabies virus grown in a stable cell line, inactivated, and mixed with a potent adjuvant.. VacciGel™ Direct Rabies Virus ELISA is intended to be used for Rabies Vaccines (Killed or subunit) that contain Rabies surface proteins adsorbed on Alum (aluminum hydroxide).

This kit is not suitable for measuring Rabies Virus proteins in solution or on non-alum formulations of the vaccines. ADI has developed other ELISA kits for the measurement of Rabies antibodies.

### Precision

Samples (n=5) were run on the same lot to determine intra-assay variability. Samples (n=5) were run across multiple days on multiple lots to determine inter-assay variability

	High	Medium	Low
<b>Inter-assay</b>	1.56%	4.12%	14%
<b>Intra-assay</b>	1.81%	2.05%	2.20%

### Suggestions for good performance for VacciGel™ ELISA

VacciGel™ ELISA is an unusual test because the antigen-antibody reaction is being performed directly on the vaccine that is in a gel suspension or precipitate. Therefore, it is very important to take a uniform amount of the sample gel and dilute it in VacciGel™ diluent (pink) in order to avoid loss of the small gel particle during prolonged processing of the test. User's must get a good understanding of the protocol and the role of each step. The VacciGel™ ELISA differs from a regular ELISA due to the nature of the samples (particulate or Alum gels). It very critical to be patient during the manual wash process and remove traces of the wash solutions without losing the sample (gel pellet). Some common issues observed:

- High standards give low values-** 1) Generally due to the loss of the standard gel pellet during the assay. 2) Not mixing the standards before taking the samples. 3) Antibody-HRP used at a lower concentration or higher dilution than the recommended 1:100.
- Standards or duplicates show high variations-** Generally one more of the issues seen in example 1
- Vaccine gel pellet too tight and does not resuspend easily** – Gel Suspension may have been spun too long or too fast. We recommend 3000 rpm for 60 seconds at each step. Lower the speed or time if the pellet is too tight.
- Vaccine gel pellet too loose and risk of loss** – Gel suspension may have been spun too short or too slow. We recommend 3000 rpm for 60 secs at each step. Increase the speed or time if a loose pellet is observed
- It is a good idea to run just the standards and a few samples to get familiar with the protocol before running too many samples to avoid unusual delays or loss of the reagents or kit.

## ELISA kits available from ADI (see details at the web site)

Catalog#	Product Description
600-020-HRV	Human Anti-Rabies Virus IgG ELISA Kit, 96 tests, Quantitative
600-030-MRG	Mouse Anti-Rabies Virus IgG ELISA Kit, 96 tests, Quantitative
600-040-RRG	Rabbit Anti-Rabies Virus IgG ELISA Kit, 96 tests, Quantitative
600-230-MRG	Mouse Anti-Rabies Virus Nucleoprotein (RV-NP) IgG ELISA Kit, 96 tests, Quantitative
600-240-RRG	Rabbit Anti-Rabies Virus Nucleoprotein (RV-NP) IgG ELISA Kit, 96 tests, Quantitative
600-220-HRV	Human Anti-Rabies Virus Nucleoprotein (RV-NP) IgG ELISA Kit, 96 tests, Quantitative
600-070-CRG	Monkey Anti-Rabies Virus IgG ELISA kit, 96 tests, Quantitative
600-140-RRG	Rabbit Anti-Rabies Virus Glycoprotein (RVG) IgG ELISA Kit, 96 tests, Quantitative
600-120-HRV	Human Anti-Rabies Virus Glycoprotein (RVG) IgG ELISA Kit, 96 tests, Quantitative
600-015-GRV	G. Pig Anti-Rabies Virus IgG ELISA Kit, 96 tests, Quantitative
600-130-MRG	Mouse Anti-Rabies Virus Glycoprotein (RVG) IgG ELISA Kit, 96 tests, Quantitative
600-170-FRG	Ferret (Fishers/Skunk) Anti-Rabies Virus Glycoprotein (RVG) IgG ELISA Kit, 96 tests, Quantitative
600-660-BRG	Monkey Anti-Rabies Virus Nucleoprotein (RV-NP) IgG ELISA kit, 96 tests, quantitative
600-095-BRG	Bat Anti-Rabies Virus IgG ELISA kit, 96 tests, Quantitative
600-180-BRG	Monkey/Baboon Anti-Rabies Virus Glycoprotein (RVG) IgG ELISA kit, 96 tests, quantitative
600-090-FRG	Ferret (Fishers/Skunk) Rabies Virus IgG ELISA kit, 96 tests, Quantitative
4220-AHB	Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) ELISA kit, Quantitative, 96 tests
VAC-DTX-50	VacciGel™ Direct ELISA for the measurement of Diphtheria Toxoid in Vaccines formulated in Alum
VAC-DTX-210	Diphtheria Toxoid/Toxin (DTX) ELISA for the measurement DTX in biological buffer
VAC-HBS-50	VacciGel™ Direct ELISA for the measurement of Hepatitis B Vaccine (HBsAg) formulated in Alum
VAC-HCG-50	VacciGel™ Direct ELISA for the measurement of HCG (contamination) in Vaccines formulated in Alum
VAC-PTX-50	VacciGel™ Direct ELISA for the measurement of Pertussis Toxoid in Vaccines formulated in Alum
VAC-PTX-410	Pertussis Toxoid/Toxin (PTX) ELISA for the measurement PTX in biological buffer
VAC-TTX-50	VacciGel™ Direct ELISA for the measurement of Tetanus Toxoid in Vaccines formulated in Alum
VAC-TTX-310	Tetanus Toxoid/Toxin (TTX) ELISA for the measurement TTX in biological buffer