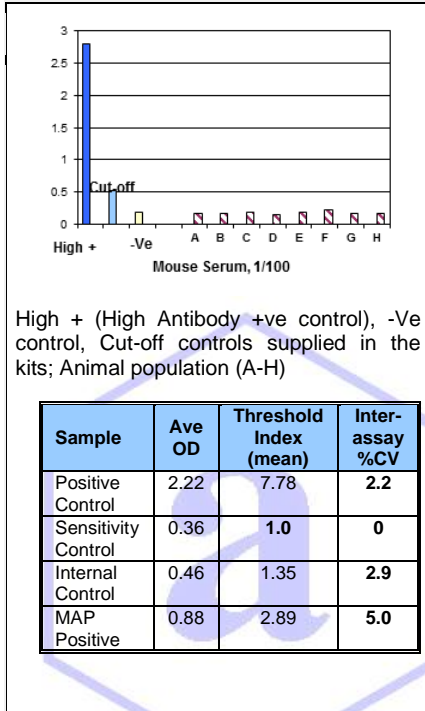


RecombiVirus™ ELISA for the quantitative detection of various animal Viruses antibodies

Conventional ELISAs (1st generation) were developed using crude viral/host cell proteins. RecombiVirus™ ELISAs are 2nd generation immunoassay using highly purified, recombinant and antigenic proteins of animal viruses. These kits are superior for enhanced sensitivity, specificity, quantitative IgG/IgM determination, smaller sample size, and safety.



RecombiVirus™ Animal Viruses Antibody ELISA Features

- Highly purified recombinant viral antigenic proteins (nucleoproteins, envelop proteins, ORFs) coated plates; Stability ~6-12 months
- No need to test samples with control host cell proteins; save precious samples and reduce cost.
- High sensitivity ELISA allow sample testing at 1:100-1:200 or higher
- Animal (mouse, rat, rabbit or g. pig virus specific antibody **positive controls** assigned at 100 u/ml (diluted to 50, 25, 12.5, 6.25 u/ml) allows quantitative antibody assay;
- Additional negative and cut-off antibody controls, 105 min RT assay.
- **RecombiVirus™ ELISAs** available for Sendai, norovirus (MNV), hepatitis virus (MHV), Polyoma virus, parvovirus (MPV), Rotavirus/EDIM, LCMV, minute virus (MVM), kilham rat virus (KRV), Toolan H-1 virus, SDAV and pneumonia virus (PVM) for mouse, rat, and G. pigs.

This kit For in vitro research use only.

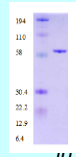


Fig 1 on the left: Top panel Mouse Anti-LCMV IgG high positive (100 u/ml) control, Cut-off control and -ve control. Mouse samples A-H, normal population of healthy mice. Bottom graph: Mouse anti-LCMV IgG standard curve. Right: Recombinant purified Mouse LCMV antigenic capsid protein (>98% pure, ~64 Kda) used to detect anti-LCMV IgG mouse sera.

All ELISA kits follow the same basic design, assay procedure etc.

General Assay Procedure: Allow all reagents to reach room temperature. Arrange and label required number of tubes.

- Step 1.** Pipet 50 ul each of pre-diluted quantitative positive standards (6.25-100 u/ml), and samples (1:100 or higher).
- Step 2.** Mix gently for 5-10 seconds and incubate for 60 min at room temp.
- Step 3.** Wash 3X using supplied wash buffer. Add 50 ul of Antibody-HRP Conjugate to all wells, mix by gentle mixing for 5-10 seconds and incubate at room temperature for 30 min.
- Step 4.** Wash 4X using supplied wash buffer. Add 50 ul of Substrate solution to all wells, mix gently, and incubate at room temperature for 15 min. Blue color develops in standards and positive samples.
- Step 5.** Pipet 50 ul of stop solution into all tubes, mix gently (blue color turns yellow). Measure OD at A450 nm. Positive samples can be observed visually and the antibody concn calculated from the standard curve.

Summary of the advantages and disadvantages of the convention and RecombiVirus of ELISAs.

	Conventional Crude Viral Protein Extracts Based ELISA	RecombiVirus™ ELISA using purified viral, antigenic, recombinant proteins
Coating antigen	Whole virus is propagated in host cells (mammalian) and crude viral antigens (<1%) stock prepared	Highly purified (>95%) recombinant (E. coli, baculovirus, or Yeast) viral antigenic viral proteins.
ELISA format	samples evaluated on host cell proteins alone as well as host cell+viral proteins	no need to test on control host cell proteins. Saves precious samples and time as well.
Sensitivity	Low level of viral protein coating compromise sensitivity and increase background	Purified viral proteins coated at high concentration increases sensitivity and reduced background.
Specificity	Many viruses share several viral protein or have sequence conservations leading to reduced specificity	Coating antigen is carefully selected for its specificity for a given virus
Production issues	Replication of live virus requires BSL-2/3 labs and the process is labor intensive and viral yields are low.	Recombinant proteins are expressed in relatively safe host such as E. coli or yeast. No issues of virus contamination of animals or humans.
Reproducibility	Due to inherent virus propagation issues reproducibility is compromised	Recombinant proteins purified in large batches leading to more consistent and reliable tests
Antibody Quantitation Cut-Off controls	Provide only -Ve and +ve controls. No cut-off controls are provided to help determine the status of -ve or +ve. Positive are only qualitative.	Kit contains -ve, +ve controls and a cut-off control. Results can be expressed as -ve/+ve or antibody concn calculated from the standard curves.
Overall assessment	Whole viral antigen-based ELISA still offered great improvement over HAI or IFA but recombinant protein based assays are preferred.	Recombinant viral protein based ELISA offer better format, save precious sample, safe handling, high sensitivity and specificity.

Animal Virus Serology - General Information

Animals, just like humans, are susceptible to various bacterial and viral infections. Animals are used widely in biomedical research. Laboratory animal infections may compromise the health of the animals and ultimately the research data derived from them. Microbial infections alter not only the animal behavior but also the biological responses. Apart from the use of whole animals for experimentations, numerous animal cell lines and proteins are also derived from animals and used in biomedical research. Animals or animal-derived products are transported from one part of the world to another in a matter of days. So there is great potential for the diseases to spread very quickly. Many infections are asymptomatic and without any overt clinical symptoms. Detection of microbial infections has relied largely on serological screening and presence of microbial antigens or antibodies. A variety of Immunoassays (ELISA, IFA, HAI, Western blot) have been developed to detect the presence of bacterial or virus specific antigen or antibodies. ELISA offers many advantages over the other immunoassay due to simple methodology, ability to handle large number of samples in short time, relative low cost, and sensitivity of the technique. Large volumes of epidemiological data have been compiled to determine the prevalence of laboratory animal diseases due to various viruses and bacteria (**Table 1**).

ELISA, when available, is the method of choice as IFA (immunofluorescence assay) HAI (hemagglutinin immunoassay) are not only cumbersome, less sensitive, more expensive but not suitable for large number of samples. The viral antigens for the first generation of ELISA were developed using crude viral extracts prepared from virus produced in a variety of animal and human cell lines. With the advancement of recombinant DNA technology and our knowledge about the viral antigenic proteins, significant improvements have been made using recombinant viral proteins as antigens to detect virus specific antibodies. Specific viral recombinant proteins (envelop proteins, nucleoprotein or membrane proteins) have been shown to be antigenic and react with antibodies produced by the whole virus or during natural infections. There is more consistency in the production and usage of Recombinant protein-based ELISA kits than the crude viral antigens. There is also no danger of contamination of the animal colony or the personnel when using recombinant proteins. The situation is very similar to human clinical diagnostic assays for hepatitis or HIV. Now all of the tests are done using purified recombinant proteins. **ADI is the first company to commercially develop 2nd generation RecombiVirus™ ELISA kits for the detection of antibodies to various animal viruses.**

RecombiVirus ELISA are based upon purified recombinant viral proteins (Capsid Proteins/CP/VP or Nucleoproteins (NP)).

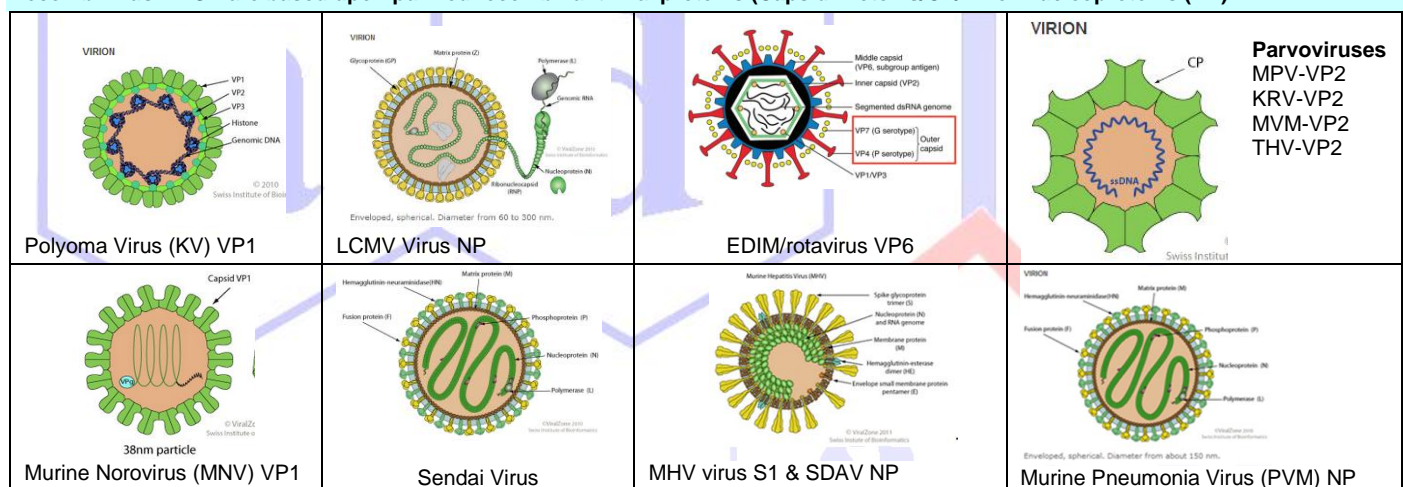


Table 1: Prevalence of viral infections in rodents (Serological screening, International Council of Laboratory Animal Science (ICLAS))

Viral Disease (abbreviation)	Virus Type	Test	Mouse	Rat	G. pig
Minute virus of mice (MVM)	ssDNA, parvovirus	ELISA	+		
Parvovirus (MPV)/Rat Kilham/Toolan's H1	ssDNA	ELISA	+	+	+
Mouse hepatitis virus (MHV)	Corona virus/ssRNA	ELISA	+		
Pneumonia virus of mice (PVM)	ssRNA/pneumovirus	ELISA	+	+	+
Murine Norovirus (MNV1)	ssRNA/calci virus/+ve Strand	ELISA	+	+	+
Sendai virus (Sev)	ssRNA/-ve strand	ELISA	+	+	+
Theiler's murine encephalomyelitis virus (TMEV)		ELISA	+	+	
Lactate dehydrogenase elevating virus (LDV/LDHV)	ssRNA, Togaviridae	Enzyme	+		
Lymphocytic choriomeningitis virus (LCMV)	ssRNA/Arenavirus	ELISA	+		+
Mouse rotavirus/ epizootic diarrhea of infant mice (EDIM)	dsRNA	ELISA	+		
Mouse K virus (K)		HAI	+		
Mouse polyomavirus KV/Pneumotropic virus	dsDNA/kilham strain/MpTV/MPyV	HAI	+		
Mouse cytomegalovirus (MCMV)		ELISA	+		
Sialodacryoadenitis/Rat coronavirus (SDA/RCV)	Corona virus/ssRNA	ELISA		+	

ELISA: enzyme linked immunosorbent assay; HAI: haemagglutination inhibition assay; IFA: immunofluorescence assay. The information is compiled from Nimejen survey (Schoondermark-van de Ven, EME (2006) 40, 137-143)

RecombiVirus ELISA kits available from ADI

Species	Catalog#	Product Description
Mouse	AE-300100-1	RecombiVirus Mouse Polyomavirus (KV, Kilham Strain/Pneumotropic virus) Antibody ELISA Kit, 96
	AE-300200-1	RecombiVirus Mouse Lymphocytic choriomeningitis virus (LCMV) Antibody ELISA Kit, 96 tests
	AE-300300-1	RecombiVirus Mouse Norovirus 1 (MNV-1) Antibody ELISA Kit, 96 tests
	AE-300400-1	RecombiVirus Mouse Epizootic diarrhea of infant mice (EDIM)/rotavirus Antibody ELISA Kit, 96 tests
	AE-300500-1	RecombiVirus Mouse Parvovirus (MPV) Antibody ELISA Kit, 96 tests
	AE-300600-1	Mouse Sendai/(SeV/Para influenza virus 1) Antibody ELISA Kit, 96 tests
	AE-300700-1	RecombiVirus Mouse Hepatitis virus (MHV/Coronavirus) Antibody ELISA Kit, 96 tests
	AE-300900-1	RecombiVirus Mouse Minute Virus (MVM) Antibody ELISA Kit, 96 tests
	AE-310400-1	RecombiVirus Mouse Pneumonia Virus (PVM) Antibody ELISA Kit, 96 tests
Rat	AE-300410-1	RecombiVirus Rat Epizootic diarrhea of infant mice (EDIM)/rotavirus Antibody ELISA Kit, 96 tests
	AE-300610-1	Rat Sendai/(SeV/Payment virus 1) Antibody ELISA Kit, 96 tests
	AE-300910-1	RecombiVirus Rat Minute Virus (MVM) Antibody ELISA Kit, 96 tests
	AE-310110-1	RecombiVirus Rat Kilham Virus (KRV) Antibody ELISA Kit, 96 tests
	AE-310210-1	RecombiVirus Rat Toolan's H-1 Virus (H-1) Antibody ELISA Kit, 96 tests
	AE-310310-1	RecombiVirus Rat sialodacryoadenitis Virus (SDAV) Antibody ELISA Kit, 96 tests
	AE-310410-1	RecombiVirus Rat Pneumonia Virus (PVM) Antibody ELISA Kit, 96 tests
G. Pig	AE-300620-1	G. Pig Sendai/(SeV/Payment virus 1) Antibody ELISA Kit, 96 tests
Hamster	AE-300630-1	Hamster Sendai/(SeV/Payment virus 1) Antibody ELISA Kit, 96 tests
Bovine	AE-300420-1	RecombiVirus Bovine Epizootic diarrhea of infant mice (EDIM)/rotavirus Antibody ELISA Kit, 96 tests
	AE-300430-1	RecombiVirus Porcine Epizootic diarrhea of infant mice (EDIM)/rotavirus Antibody ELISA Kit, 96 tests
	AE-300440-1	RecombiVirus Sheep Epizootic diarrhea of infant mice (EDIM)/rotavirus Antibody ELISA Kit, 96 tests
	AE-300450-1	RecombiVirus Monkey (Rhesus/Cyno/Chimp) Epizootic diarrhea of infant mice (EDIM)/rotavirus Antibody ELISA Kit, 96 tests
Chicken	AE-300640-1	Chicken Sendai/(SeV/Payment virus 1) Antibody ELISA Kit, 96 tests

ELISA kits for the measurement of serum Igs and major serum proteins are also available.

Serum Proteins	Mouse	Rat	Porcine/Pig	Monkey	Bovine	Chicken	Rabbit	Human
IgA	6310			7010				1780
IgGs (total)*	6320	6420	9020	7050	8010	6020	6520	1750
IgM	6380	6480	9080	7060	8080	6040		1760
IgG1	6330							1710
IgG2a	6340							
IgG2b	6350							
IgG3	6360							
IgG4								1740
IgE	6370			7070				1800
Albumin	6300	6400	9000		8000 & 8100			1190

IgG* (total)-This kit measure total IgG (mixture of all IgGs or isotypes but not IgA, IgM, IgE etc). IgG1 for example measures only IgG1 isotype and not IgG2, IgG3 etc.

Autoimmune diseases (anti-dsDNA/ssDNA/ANA/Histones etc) ELISAs for mouse, rat, human, and other species are also available. Please see details of these ELISA kits using specific cat# or contact ADI for additional details. 121212A