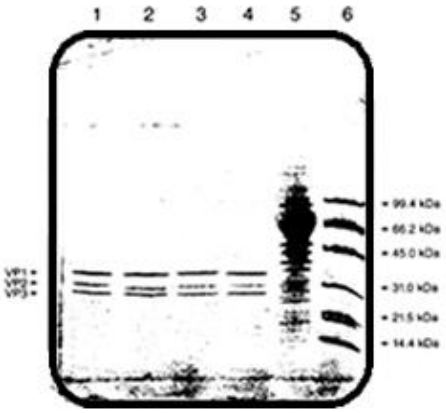
BIOTECH SUPPORT GROUP

ViraPrep™ Mammal

Virus and Viral Component Isolation Kit for Mammalian Viruses

- Protocols less than 90 minutes
- Kits contain all conditioning and elution buffers for binding and elution of viruses
- Compatible with detergents and chaotropes
- No ultracentrifugation required



Chimeric Rhinovirus Isolation

Lanes 1 & 2: ViraPrep™ purified Chimeric Rhinovirus.
Lanes 3 & 4: Sucrose Gradient purified Chimeric Rhinovirus.
Lane 5: Lysate of infected cells, sample prior to ViraPrep™ protocol.
Lane 6: Molecular weight marker

Product	Processes up to:	Item No.
ViraPrep™ Mammal	40 ml total virus sample volume	VPM-40

ViraPrep™ Mammal is a kit that adapts the unique Viraffinity™ reagent for virus isolation. Viraffinity™ is a suspension reagent containing a water-insoluble elastomeric polyelectrolyte that has been engineered for the capture and recovery of viruses.

Items Required	Quantity	ViraPrep™ Mammal	Storage
06CB1 Conditioning 0.06 M Buffer	160 ml	Supplied	Refrigerate.
1CB1C Conditioning 1 M Buffer	2 ml	Supplied	Refrigerate.
EB2 Elution Buffer	120 ml	Supplied	Refrigerate.
Viraffinity™	15 ml	Supplied	4°C

The virus-containing sample is first conditioned to pH 6.5 using the enclosed "Conditioning Buffer". After resuspending the solid-phase, Viraffinity™ is added to the sample. After mixing, the sample is centrifuged to pellet the polymer plus the adsorbed viruses. The polymer pellet is then washed further. To recover the virus or viral antigens, the pellet is washed and resuspended in a moderately alkaline pH, the enclosed "Elution Buffer" EB2.

Viraffinity™ Performance Characteristics (partial list)

Virus	Titer	Ratio	% Bound[b]
HIV-1[a]	$7x10^3$ $TCID_{50}$	1 : 2	96
HIV-1, human serum	$7x10^3$ $TCID_{50}$	1 : 2	80
Chimeric Human Rhinovirus[a]	10^6-10^8 pfu/ml	1 : 3	95
Dengue virus	After Sucrose density Ultra-centrifugation	1:2	
Adenovirus (Ad5d1309)[a]	10^6-10^8 pfu/ml	1 : 3	90
Reovirus Type 3[a]	10^6-10^8 pfu/ml	1 : 3	50-80
Encephalomyocarditis (EMC)[a]	10^7 $TCID_{50}$	1 : 4	99
Porcine Parvovirus[a]	10^7 $TCID_{50}$	1 : 2	90
Unclassified Entero-Picornavirus[a]	10^6 $TCID_{50}$	1 : 4	90
Coxsackievirus A24[a]	10^6-10^7 $TCID_{50}$	1 : 2	70-95
Bacteriophage Lambda	10^9 pfu/ml	1 : 5	>95

Ratio refers to the volumetric ratio of Viraffinity™ to unconditioned sample.
[a] Tissue culture supernatants containing 1 - 10% Fetal Bovine Serum
[b] based on infectivity

STORAGE

Viraffinity™ is supplied as an aqueous suspension of a water-insoluble polyelectrolyte in buffer. The reagent should be kept sealed and stored at 4°C. Do not freeze. Viraffinity retains full activity when stored accordingly for approximately 1 year.

PROTOCOL

To date, viability of enveloped viruses after desorption of the virus has been anecdotal only. However, viability has been demonstrated when the viruses are non-enveloped although optimization for any given virus has been limited. In all cases, the recovery of virions, viral proteins or viral nucleic acids are good applications for ViraPrep™.

This protocol can be used for cell culture supernatants, clarified cell culture lysates, serum, plasma, cervical fluid, biological extracts or other types of samples.

1. For serum, plasma, cervical fluid, biological extracts or other types of concentrated samples, add 1 volume of "**06CB1**" to sample. Alternatively, for clarified cell culture samples, condition with 1 volume of "**1CB1**" to 30 volumes sample.

2. Incubate with 1:4 volume ratio, **Viraffinity**™:sample, based on initial sample volume. If necessary, the ratio can be adjusted according to the titer of sample, a minimum ratio of 1:5 **Viraffinity**™:sample, however, is recommended for quantitative recovery.

3. Mix well and let stand for 5 minutes at room temperature.

4. Pellet by centrifugation, 1,000 X G for 10 minutes. NOTE: Do not use maximum G-force to pellet as it makes subsequent steps difficult to re-suspend.

5. Decant and discard supernatant and wash the pellet with the equivalent starting volume of the sample. Use conditioning buffer **06CB1** for all washes. Repeat washing and pelleting steps 2 more times.

6. To recover virus, resuspend the pellet in 1 to 3 volumes (based on initial sample size) of "**EB2**" buffer. For highest recovery, use 3 volumes; for minimal dilution, use 1 volume.

   Optionally, a detergent such 1% N-lauroyl sarcosine may be added to aid in recovery and purity.

7. Pellet by centrifugation using maximum G-force for 10 minutes. Recover supernatant.

Viraffinity™ References

Dengue Virus

Romain Fragnoud, Marie Flamand, Frederic Reynier, Philippe Buchy, Vasna Duong, Alexandre Pachot, Glaucia Paranhos-Baccala and Frederic Bedin. **Differential proteomic analysis of virus enriched fractions obtained from plasma pools of patients with dengue fever or severe dengue.** BMC Infectious Diseases (2015) 15:518. http://www.ncbi.nlm.nih.gov/pubmed/26572220

Norovirus

Shinohara, Michiyo, Kazue Uchida, Shin-ichi Shimada, Kyoko Tomioka, Noriko Suzuki, Toshitaka Minegishi, Sachie Kawahashi, Yuko Yoshikawa, and Norio Ohashi. Application of a simple method using minute particles of amorphous calcium phosphate for recovery of norovirus from cabbage, lettuce, and ham.Journal of Virological Methods (2012)

Hanta Virus

Hai-Tao Yu, Hong Jiang, Ye Zhang, Xue-Ping Nan, Yu Li, Wei Wang, Wei Jiang, Dong-Qiang Yang, Wen-Jing Su, Jiu-Ping Wang, Ping-Zhong Wang, and Xue-Fan Bai.Hantaan Virus Triggers TLR4-Dependent Innate Immune Responses. Viral Immunology.2012;

Mihalic, K. A., et al, Development of a Chemiluminescent Western Blot for Detecting Hantaan-Specific Antibodies, poster American Society of Tropical Medicine and Hygiene Meeting, October 1997.

Human Mouth Virus

Metagenomic Analysis of the Human Mouth Virus Population and Characterisation of Two Lytic Viruses, Al-Jarbou, Ahmed, Theses, Dept. of Infection. Immunity and Inflammation.2009

Lepidoptera: Gelechiidae

An isometric virus of the potato tuber moth Tecia solanivora (Povolny) (Lepidoptera: Gelechiidae) has a tri-segmented RNA genome Jean-Louis Zeddam et. al Journal of Invertebrate Pathology, Volume 99, Issue 2, October 2008, Pages 204-211

Human Immunodeficiency Virus Type 1

Activity of the Small Modified Amino Acid -Hydroxy Glycineamide on In Vitro and In Vivo Human Immunodeficiency Virus Type 1 Capsid Assembly and Infectivity Antimicrobial Agents and Chemotherapy, October 2008, p. 3737-3744, Vol. 52, No. 10 Samir Abdurahman

Mutation in the loop C-terminal to the cyclophilin A binding site of HIV-1 capsid protein disrupts proper virus assembly and infectivity Samir Abdurahman , Stefan Höglund , Anders Höglund and Anders Vahlne Retrovirology 2007, 4:19doi:10.1186/1742-4690-4-19

Poliovirus type 1 (PV1), Hepatitis A virus (HAV), Norwalk virus

Detection Methods for Human Enteric Viruses in Representative Foods. Leggitt, Paris R.1; Jaykus, Lee-Ann, Journal of Food Protection®, Volume 63, Number 12, December 2000 , pp. 1738-1744(7).

Polio Virus

Ting, W.T. E., E. M. Nielson, and C.C. Tseng. 1997. The use of Viraffinity matrix to concentrate waterborne polioviruses for RT-PCR detection. Abstr. Q-169. p.484. InAbstracts of the 97th General Meeting of the American Society for Microbiology 1997, American Society for Microbiology, Washington, D.C.

CONTACT US

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