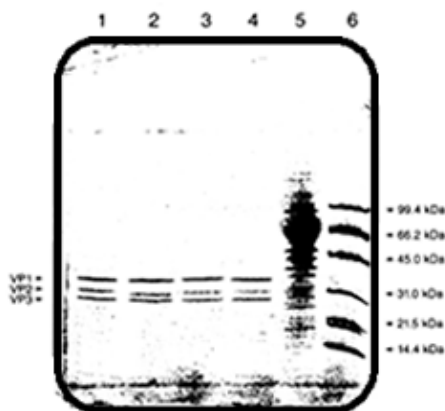


ViraPrep™ Mammal

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	400 ml total virus sample volume	VPM-400

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	Room Temp.
1CB1C Conditioning 1 M Buffer	2 ml	Supplied	Room Temp.
EB2 Elution Buffer	120 ml	Supplied	Room Temp.
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.

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
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 Enteric 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.
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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.

REFERENCES

1. Composition and utility patents for Viraffinity™ and related technologies are covered under U.S. Patent Numbers 5,294,681, 5,453,493 & 5,658,779 and other patents pending.
2. 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.
3. Hitti, J., et al, *Fast and Convenient Purification of Bacteriophage Lambda DNA with Viraffinity™ Matrix*, poster Cold Spring Harbor Conference on Genome Mapping & Sequencing, May 1997.
4. Ting, W. T. E., et al, *The Use of Viraffinity™ Matrix to Concentrate Waterborne Polioviruses For RT-PCR Detection*, poster American Society of Microbiology, May 1997.
5. Lee, A., et al, *Rapid Detection of Foodborne Viruses from Minimally Processed Foods*, poster Australian Society of Microbiology, July 2000.
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