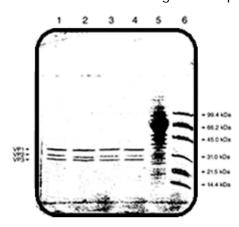


# ViraPrep™ Mammal

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<sup>™</sup> 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<sup>™</sup> Mammal is a kit that adapts the unique Viraffinity<sup>™</sup> reagent for virus isolation. Viraffinity<sup>™</sup> 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 I	ist)	)
--	------	---

Virus	Titer	Ratio	% Bound <sup>b</sup>
-------	-------	-------	----------------------

HIV-1 <sup>a</sup>	7x10 <sup>3</sup> TCID <sub>50</sub>	1:2	96
HIV-1, human serum	7x10 <sup>3</sup> TCID <sub>50</sub>	1:2	80
Chimeric Human Rhinovirus <sup>a</sup>	10 <sup>6</sup> -10 <sup>8</sup> pfu/ml	1:3	95
Adenovirus (Ad5d1309) <sup>a</sup>	10 <sup>6</sup> -10 <sup>8</sup> pfu/ml	1:3	90
Reovirus Type 3 <sup>a</sup>	10 <sup>6</sup> -10 <sup>8</sup> pfu/ml	1:3	50-80
Encephalomyocarditis (EMC) <sup>a</sup>	10 <sup>7</sup> TCID <sub>50</sub>	1:4	99
Porcine Parvovirus <sup>a</sup>	10 <sup>7</sup> TCID <sub>50</sub>	1:2	90
Unclassified Entero-Picornavirus <sup>a</sup>	10 <sup>6</sup> TCID <sub>50</sub>	1:4	90
Coxsackievirus A24 <sup>a</sup>	10 <sup>6</sup> -10 <sup>7</sup> TCID <sub>50</sub>	1:2	70-95
Bacteriophage Lambda	10 <sup>9</sup> pfu/ml	1 : 5	>95

Ratio refers to the volumetric ratio of Viraffinity™ to unconditioned sample.

### **STORAGE**

Viraffinity<sup>™</sup> is supplied as an aqueous suspension of a water-insoluble polyelectrolyte in buffer. The reagent should be kept sealed and stored at  $4^{\circ}$ C. Do not freeze. Viraffinity<sup>™</sup> 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 $^{\text{TM}}$ .

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 "O6CB1" 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 **O6CB1** for all washes. Repeat washing and pelleting steps 2 more times.

<sup>&</sup>lt;sup>a</sup> Tissue culture supernatants containing 1 - 10% Fetal Bovine Serum

b based on infectivity

- 6. <u>To recover virus</u>, 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

#### **Human Mouth Virus**

Al-Jarbou, Ahmed N. "Metagenomic Analysis of the Human Mouth Virus Population and Characterisation of Two Lytic Viruses." PhD diss., University of Leicester, 2008.



Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, BELGIUM
Tel 0032 16 58 90 45 | Fax 0032 16 50 90 45
www.gentaur-worldwide.com
info@gentaur.com