Viraffinity™

Virus and Viral Component Isolation

- Purifies whole infectious non-enveloped virus & non-infectious enveloped virus
- Isolates antigenic virions, enveloped and non-enveloped
- Enriches for viral nucleic acids
- Prepares viral samples for subsequent detection and analysis

Viraffinity™ is a unique water-insoluble elastomeric polyelectrolyte that has been engineered for the capture and recovery of viruses. Applications include: purification of whole infectious non-enveloped virus, virions, viral components, and sample preparation for subsequent detection and analysis. Viraffinity™ is directly added to the sample that is then mixed and centrifuged. The centrifuged pellet contains polyelectrolyte-bound viruses that can then be recovered using a moderately alkaline pH solution.

Viraffinity™ is supplied as a suspension reagent ready for use. Simply pipette the suspension into the sample at the appropriate ratio, typically 1 volume of Viraffinity™ to 4 volumes sample. Viraffinity™ is also supplied as the enabling component of the ViraPrep™ application kits, see following page.

Product	Size	Item No.
Viraffinity™ 15	**15ml**	**V1062-15**
Viraffinity™ 50	**50ml**	**V1062-50**
Viraffinity™ 100	**100ml**	**V1062-100**

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 Entero-Picornavirus [a]	$10^6 TCID_{50}$	1:4	90
Coxsackievirus A24 [a]	10^6 - 10^7 pfu/ml	1:2	70-95
Bacteriophage Lambda	10^9 pfu/ml	1:5	>95

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

[a] Tissue culture supernatants containing 1-10% Fetal Bovine Serum.

[b] Based on infectivity.

Storage

Supplied as an aqueous suspension of a synthetic, anionic 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

Purification of Infectious Virus, Virions and Viral Components
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 Viraffinity™.

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. Add 1 volume of 60 mM MES, 150 mM NaCl, pH 6.5 to sample. Alternatively, for clarified cell culture samples, condition with 1:30 volume of 1 M MES, pH 6.5.

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 60 mM MES, 150 mM NaCl, pH 6.5 for all washes. Repeat washing and pelleting steps 2 more times.

6. To recover and maintain viable non-enveloped virus, resuspend the pellet in 1 to 3 volumes (based on initial sample size) of 100 mM Tris-Base, 100 mM Borate pH 9.0. Optionally, a detergent such 1% N-lauroyl sarcosine may be added to aid in recovery and purity.

   To recover virions or viral proteins, resuspend the pellet in 1 to 3 volumes (based on initial sample size) of elution buffer to pH 10.5. Optionally, detergents such as Triton or SDS can be used in conjunction with the buffer to isolate viral proteins.

   To recover viral nucleic acids, resuspend the pellet in 1 to 3 volumes (based on initial sample size) of a lysis buffer of the users choice. These may contain chaotropes or detergents such as Triton or SDS which are compatible with Viraffinity™. Temperatures to 65°C may also be used to assist with lysis.

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

Mammalian Virus & Lambda DNA Isolation Kits

ViraPrep™ Mammal contains **Viraffinity™** and all necessary buffers and protocols for mammalian virus and virion isolation. **ViraPrep™Lambda** is a complete application kit containing Viraffinity™ and all the necessary reagents for obtaining high purity DNA from plate or liquid lysates, suitable for amplification, automated fluorescent sequencing and other common molecular biology techniques. Please inquire.

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.
6. P.R. Leggitt, L. Jaykus, *Detection Methods for Human Enteric Viruses in Representative Foods*, Journal of Food Protection, Vol. 63, No. 12, 2000, p. 1738-1744.