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Viraffinity™

Virus and Viral Component Isolation

- Purifies whole infectious non-enveloped virus & non-infectious enveloped virus
- Prepares viral samples for subsequent detection and analysis
- Alternative or complementary to ultracentrifugation
  - "…love this vs sucrose cushions!" Maggie L. Bartlett, BS PhD, Johns Hopkins Bloomberg School of Public Health
  - or can complement ultracentrifugation, for viral proteomic enrichment 350X (Fragnoud et al, 2016 http://www.ncbi.nlm.nih.gov/pubmed/26572220)
- Isolates antigenic virions, enveloped and non-enveloped
- Enriches for viral nucleic acids; detergent, chaotrope, Trizol® compatible

Viraffinity™ is a unique water-insoluble elastomeric polyelectrolyte that has been engineered for the capture and recovery of viruses. It is supplied as a suspension reagent ready for use. 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 a pre-conditioned sample, is then mixed and centrifuged using standard micro-centrifuges (not ultra). The centrifuged pellet contains polyelectrolyte-bound viruses that can then be recovered using a moderately alkaline pH solution.

Related Viraffinity™ Kits

ViraPrep™ Mammal contains **Viraffinity™** and all necessary buffers and protocols for mammalian virus and virion isolation.
https://www.biotechsupportgroup.com/ViraPrep-Mammal-p/vpm.htm

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.
https://www.biotechsupportgroup.com/ViraPrep-Lambda-p/vlk.htm

Product	Size	Item No.
Viraffinity™ 15	15ml	V1062-15

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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]	10^7TCID_{50}	1:4	99
Porcine Parvovirus [a]	10^7TCID_{50}	1:2	90
Unclassified Entero-Picornavirus [a]	10^6TCID_{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 original sample volume.
[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.

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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. Consider the 3 recovery options described in (6) before proceeding.
6. A. 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.

   6B. 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. As an option to recover viral proteins for proteomic analysis, 5 min at 70 °C in SDS-buffer can be used.

   6C. 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, detergents (i.e., Triton or SDS), or Trizol® which are all 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.

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.

Bacteriophage Lambda DNA

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.

Related ViraPrep™ 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.

Related Molecular & Micro-biology products

ProCipitate™ - Superior Substitute to Phenol/Chloroform for Hemoglobin & Total Protein Removal, Isolation of DNA/RNA
Cleanascite™ for Lipid Removal & Sample Clarification
https://www.biotechsupportgroup.com/Articles.asp?ID=455

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