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ViraPrep™ Lambda

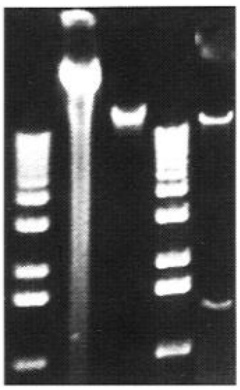
Lambda DNA purification kit with Viraffinity™

ViraPrep™ Lambda is a complete DNA purification system based upon the unique virus binding reagent, Viraffinity™. Bacteriophage is selectively captured intact from plate (or liquid) lysates within the polymer matrix. It is then pelleted and washed free of contaminants. By changing the buffer, the phage is lysed releasing the DNA while phage coat proteins and Lambda Exonuclease remain bound. The polymer is easily separated and the purified Lambda DNA is concentrated by alcohol precipitation. The entire protocol is complete in less than $1^1/_2$ hours.

The ViraPrep™ Lambda system utilizes Viraffinity™ to produce purified Lambda DNA which is suitable for endonuclease digestion, sequencing*, Southern blotting, PCR, and genomic library construction. *(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)

BENEFITS

- Non-hazardous
- Fast
- High Yield
- Simple to Use
- Scaleable



M 1 2 3

Lane M: Markers
Lane 1: PEG/Phenol-Chloroform
Lane 2: *ViraPrep*™ method
Lane 3: *Eco* RI digest of *ViraPrep*™
Note: Insert band approx. 1 kb

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Product	Size	Item No.
ViraPrep™ Lambda 05	5, 150 mm plates	VLK-05

Performance Characteristics

Culture Conditions	Titer	DNA Yield	% Bound
150mm plate lysate, solubilized in 10ml ViraPrep™ Lambda HS1 buffer, and clarified	10^9 pfu/ml (approximate)	10 - 20 μg	>95
10ml liquid lysate, plus addition of LL1 buffer, and clarified	10^9 pfu/ml (approximate)	10 - 20 μg	>95

Items Required	Quantity	ViraPrep™Lambda	Storage
LL1, Buffer (for liquid lysates)	500 ul	Supplied	4°C
HS1, Solubilization Buffer (for plate lysates)	150 ml	Supplied	4°C
RNase Cocktail	1 ml	Supplied	-20°C
HL2, Lysis Buffer	10 ml	Supplied	4°C
AA3, Ammonium Acetate	1 ml	Supplied	4°C
V1062, Viraffinity™	10 ml	Supplied	4°C
Ethanol		Not Supplied	--
Growth Media		Not Supplied	--
Final Resuspension Buffer		Not Supplied	--

CULTURE PREPARATION FOR 100MM PLATES

1. Grow Phage on 100 m plate to confluent lysis at 37°C.
2. Add 10mls of buffer HS1 and incubate 30 minutes to solubilize phage.
3. Scrape top agar into a 50ml centrifuge tube.
4. Centrifuge lysate @ 4500 rpm (Sorvall or equiv.) for 10 minutes.
5. Transfer clarified supernatant to a 15ml tube.

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LIQUID LYSATE PREPARATION

1. Centrifuge lysate @ 4500 rpm (Sorvall or equiv.) for 10 minutes.
2. Transfer 10MLS OF clarified supernatant to a 15ml tube.
3. Add 100ul of LL1 and invert 10 minutes.

PROTOCOL

1. Shake Viraffinity™ well or vortex to insure that it is completely resuspended before use. Add 2 mls of Viraffinity™ matrix to the lysate. Invert 10 times to mix and incubate at room temperature for 5 minutes.
2. Centrifuge @ 2000 rpm for 10 minutes to pellet the phage-matrix complex.
3. Aspirate and discard supernatant.
4. Wash the pellet with 10 mls of buffer HS1. A quick vortex will thoroughly resuspend the pellet.
5. After resuspension, add 200 ul RNase with a second HS1 wash and incubate @ 37°C for 15 minutes prior to centrifugation.
6. Centrifuge @ 2000 rpm for 10 minutes after each wash, remove the supernatant. The intact phage particle is contained within the pellet.
7. Resuspend the pellet in 2 mls of HL2 lysis buffer.
8. Heat @ 65°C for 10 minutes to release the DNA.
9. Centrifuge @ maximum speed for 10 minutes.
10. Remove the supernatant containing the DNA and transfer to a clean tube.
11. Add 200 ul of AA3 buffer and mix well.
12. Add 5.5 mls of Ethanol. Mix well and let stand for 10 minutes.
13. Centrifuge @ maximum speed for 10 minutes to pellet precipitated DNA.
14. Decant supernatant, air dry or speed vac the pellet.
15. Resuspend in 50 – 100 ul of TE or other buffer.
16. Check DNA purity by gel electrophoresis. Digest with appropriate restriction enzymes.

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.

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CONTACT US

We welcome your questions and comments regarding our products.

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