IG® Lambda Packaging Extract

Manual

Catalog #	3312	3314
Package Size	6 reactions	24 reactions

Custom formats and package sizes are available upon request

Important!

-80°C Storage Required

* Immediately inspect packages
* Freezer

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Intact Genomics, Inc.

Intact Genomics®

IG® Lambda Packaging Extract

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Description:

IG® Lambda Packaging Extract is a high-efficiency in vitro packaging system for lambda, cosmid, and fosmid DNA. The extract is optimized for efficient packaging of both methylated and unmethylated cos-containing DNA, making it ideal for genomic library construction, cDNA library generation, and cloning of highly modified DNA. IG® Lambda Packaging Extract routinely achieves packaging efficiencies exceeding 1×10^9 plaque-forming units (pfu) per microgram of ligated Lambda Control DNA, providing excellent library representation and reproducible performance across a wide range of applications. Its simplified protocol does not require premixing of separate extract components before use.

Unlike traditional lambda packaging systems, which are prepared from complementary lysogenic E. coli strains BHB2690 and BHB2688, IG® Lambda Packaging Extract utilizes the improved NM759 packaging strain described by Gunther et al. (1993). This restriction-deficient, K-12-derived strain lacks λ-phage capsid protein D and replaces BHB2690 in the preparation of the sonication extract. When combined with the complementary freeze-thaw extract derived from strain BHB2688, which lacks λ-phage capsid protein E, the system provides exceptionally efficient packaging of λ DNA.

The restriction-deficient design of the extract significantly improves the recovery of methylated λ DNA, including DNA carrying mammalian methylation patterns. This enhancement has been shown to increase the efficiency of rescuing λ shuttle vectors from transgenic mouse DNA and other highly modified genomic DNA, where conventional packaging extracts often exhibit reduced performance.

Applications:

- Construction of large-insert genomic libraries
- *In vitro* packaging of lambda, cosmid, and fosmid DNA
- Packaging of methylated or unmethylated DNA
- Rescue of lambda shuttle vectors from mammalian or transgenic mouse DNA
- Transfer, amplification, and screening of **cos** site-containing recombinant DNA

Benefits:

- High-efficiency packaging of lambda, cosmid, and fosmid DNA
- Packaging efficiencies of up to 1×10^9 pfu/µg DNA
- Efficient packaging of both methylated and unmethylated DNA
- Restriction-deficient system minimizes degradation of methylated DNA
- Restriction-deficient extract for improved recovery of methylated DNA
- Ideal for genomic library construction, cDNA libraries, and large-insert cloning
- Simplified workflow with no extract-component premixing required

Shipping Condition:

Ship on dry ice. Stable ONLY with dry ice shipping.

Product Components and Storage Temperature:

The following reagents are supplied with this product:

IG Cat #	Package Size	Component Name	IG Part #	Amount	Storage
3312	6 Reactions	IG® Lambda Packaging Extract	P241238	3 × 200 µL	-80 °C
		Fosmid Replicator Plating Strain (Glycerol Stock)	P241242	1 × 250 µL	-80 °C
3314	24 Reactions	IG® Lambda Packaging Extract	P241238	12 × 200 µL	-80 °C
		Fosmid Replicator Plating Strain (Glycerol Stock)	P241242	1 × 250 µL	-80 °C

Custom formats and package sizes are available upon request.

Protocols:

Plating Bacteria Preparation:

1. The day before performing the packaging reactions, inoculate 50 mL of supplemented (10 mM $MgSO_4$) LB broth with a single colony of the plating bacterial strain and shake overnight at 37 °C.
2. The day of the packaging reactions, inoculate 50 mL of supplemented (10 mM $MgSO_4$ + 0.2% maltose) LB broth with 5 mL of the overnight culture and shake at 37 °C to an OD600 = 0.8-1.0. Store the cells at 4 °C until needed; cells may be stored for up to 72 hours.

Preparation of Lambda Packaging Phage:

1. Thaw the appropriate number of packaging extracts on ice. For every two packaging reactions, thaw one extract then place on ice. Note: Ensure that the extracts are completely thawed before use.
2. When thawed, immediately transfer half (50 µL) of each packaging extract to a second 1.5-mL tube and place on ice.
3. Add the substrate DNA (upto 10 µL [0.25 µg] of cloned fosmid DNA) to a tube containing 50 µL of extract. If performing an odd number of packaging reactions, the remaining 50 µL of extract can be refrozen at –80 °C.
4. Mix by pipetting several times; avoid the introduction of air bubbles. Return all of the contents to the bottom of the tube by brief centrifugation if necessary.
5. Incubate the reaction(s) at 30 °C for 90 minutes.
6. At the end of this incubation, add the additional 50 µL of thawed extract to each reaction tube at 30 °C (If performing only one packaging reaction, returned 100 µL of remaining extracts to –80 °C) and incubate the reaction(s) for an additional 90 minutes at 30 °C.
7. Add 1,000 µL of phage dilution buffer and mix by gentle vortexing.
8. Add 50 µL of chloroform and mix by gentle vortexing (store at 4 °C).
9. Assay the packaged phage by titering on the appropriate bacterial strain (for example IG® fosmid replicator strain).

Media and Solutions:

- **LB Broth (1 Liter):** 10 g Bacto-tryptone, 5 g Bacto-yeast extract, 10 g NaCl, Adjust pH to 7.0 with NaOH
- **LB Plates:** LB Broth with 1.5% (w/v) Bacto-agar
- **LB Top Agar:** LB Broth with 0.7% (w/v) Bacto-agar

Titering Phage Extracts:

1. Make serial dilutions of the packaged phage in phage dilution buffer. Use 10^2, 10^4 and 10^6 dilutions for the control reactions.
   - 10^2 dilution is 10 µL of packaged phage particles into 990 µL of phage dilution buffer; vortex mix.
   - 10^4 dilution is 10 µL of 10^2 dilution into 990 µL phage dilution buffer; vortex mix.
   - 10^5 dilution is 100 µL of 10^4 dilution into 900 µL phage dilution buffer; vortex mix.
   - 10^6 dilution is 10 µL of 10^4 dilution into 990 µL phage dilution buffer; vortex mix.
2. Add 100 µL of the appropriate serial dilutions to 100 µL of prepared plating bacteria and incubate for 15 minutes at 37 °C.
3. Add 3.0 mL of melted LB top agar, supplemented with 10 mM $MgSO_4$ and cooled to ~48 °C. Vortex gently and pour onto pre-warmed (37 °C) LB plates. Allow the top agar to solidify and then incubate inverted overnight at 37 °C.
4. Count the plaques and determine the titer (pfu/mL) and packaging efficiency (See sample calculations).

Titer calculations:

[(# of plaques) (dilution factor) (1000 µL/mL)]/[(volume of diluted-phage plated [µL]) (amount of DNA packaged)]

If 100 plaques are observed on the plate containing the 10^6 dilution, the packaging efficiency is calculated as follows: (100 pfu) (10^6) (1000 µL/mL) /(100 µL) (0.25µg) = 4×10^9 pfu/µg of DNA.

Therefore, the packaging efficiency of this reaction is 4×10^9 pfu/µg DNA.

References:

1. Hohn, E.G. (1979) Methods Enzymol. 68, 299.
2. Gunther, E.G. et al., (1993) Nucl. Acids Res. 21, 3903.
3. Kohler, S.W. et al., (1990) Nucl. Acids Res. 18, 3007.

Related Products:

- IG® Fosmid cloning kit (custom-made)
- ig® 5-Alpha Chemically Competent Cells (Cat.# 1031-12)
- BL21(DE3) Electroporation Competent Cells (Cat.# 1212-12)
- T4 DNA Ligase (Cat.# 3212)
- i7® High Fidelity DNA Polymerase (Cat.# 3254)
- igFusion™ Cloning Kit (Cat.# 4111)

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  Email: **sales@intactgenomics.com**
  Phone: (314) 942-3655 | Toll-free : 855-835-7172 | Fax: (314) 942-3656
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Our hours are Monday - Friday, 8AM to 5PM, U.S. Central Standard Time.

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