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MaxPlax™ Lambda Packaging Extracts

Cat. Nos. MP5105, MP5110, and MP5120

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1. Introduction

MaxPlax™ Lambda Packaging Extracts are a convenient, high-efficiency system designed for in vitro lambda packaging reactions. MaxPlax Lambda Packaging Extracts are supplied as predispensed single-tube reactions that have been optimized for packaging of methylated and unmethylated DNA. The packaging extracts routinely yield packaging efficiencies of >1 x 10^9 pfu/µg of Control λ DNA. The extracts can be used in the construction of representative cDNA libraries and genomic cloning of highly modified (methylated) DNA into λ-phage or cosmid vectors.

Traditional packaging extracts are derived from two complementary lysogenic *E. coli* strains, BHB2690 and BHB2688, as described by Hohn (1979).[1] The MaxPlax extracts utilize a new packaging strain, NM759*, reported by Gunther, Murray and Glazer (1993).[2] This strain, which replaces strain BHB2690 in the preparation of the sonication extract, is a restriction-free K12-derived strain deficient in the production of λ-phage capsid protein D. When combined with the complementary freeze-thaw extract from strain BHB2688**,[1] deficient in the production of λ-phage capsid protein E, an extremely high-efficiency of packaging for λ DNA is obtained. Moreover, the ability to package λ DNA bearing the mammalian methylation pattern is greatly enhanced, as evidenced by the high efficiency of λ-vector rescue from transgenic mouse DNA.[2] The lack of restriction activity has been shown to be crucial for the high efficiency rescue of lambda shuttle vectors from transgenic mouse DNA.[2,3]

**NM759: [W3110 recA56, Δ(mcrA) e14, Δ(mrr-hsd-mcr), (λimm434, clts, b2, red3, Dam15, Sam7)/λ]*

***BHB2688: [N205 recA⁻, (λimm434 clts, b2, red3, Eam4, Sam7)/λ]*

2. Kit Contents

Desc.	Concentration	Quantity
MaxPlax Lambda Packaging Extracts are available in 5-10-, and 20-extract sizes.		
The 20-extract size kit contains the following reagents:		
MaxPlax Lambda Packaging Extracts	50 µl/tube	20 tubes
Ligated Lambda Control DNA (λc1857 *Sam7*)	(1 µg @ 0.02 µg/µl)	50 µl
Control plating *E. coli* strain LE392MP, glycerol stock		250 µl
[F⁻ e14⁻(McrA⁻) Δ(*mcrC-mrr*) (Tet^R) *hsd*R514 *sup*E44 *sup*F58 *lac*Y1 or Δ(*lac*IZY)6 *gal*K2 galT22 *met*B1 *trp*R55 λ⁻]		

Note: *MaxPlax Lambda Packaging Extracts are supplied as freeze-thaw/sonicate extracts in unlabeled single tubes. The extracts, Ligated Lambda Control DNA, and Control Plating Strain LE392MP are packaged together in a CO_2-impermeable foil pouch.*

Store the MaxPlax Lambda Packaging Extracts at –70°C or below. Exposure to higher temperature will decrease packaging efficiencies.

3. Product Specifications

Storage: Store the control host bacteria and the MaxPlax Lambda Packaging Extracts at –70°C. Exposure to higher temperatures will greatly compromise packaging extract efficiency. Avoid long term storage of product in the presence of dry ice. Once removed from the foil package, avoid any exposure to dry ice. Store the remainder of the kit components at –20°C. After thawing, store the Control DNA at 4°C.

Storage Buffers: MaxPlax Lambda Packaging Extracts are supplied as unlabeled single tubes of freeze-thaw/sonicate extracts. Control plating bacteria are supplied as a glycerol stock. Control ligated λ DNA is supplied in 1X Ligation Buffer.

Guaranteed Stability: MaxPlax Lambda Packaging Extracts are guaranteed to maintain a packaging efficiency of >1.0 x 10^9 pfu/µg of control λ DNA, when stored as directed for one year from the date of purchase.

4. Protocol for Packaging Lambda DNA

This protocol can be used for the positive control reaction as well as for experimental reactions. The positive control reactions must be plated on the control host bacterial strain (LE392MP) included with the MaxPlax Extracts. The proper bacterial plating strain for the experimental reactions will vary depending on the cloning vector used. See the vector manufacturer's recommendations for the proper strain and plating media requirements. Ligation reactions may be added directly to the packaging extracts. When doing so, it is important to: a) add a volume of 10 µl or less to the packaging reaction, and b) heat inactivate the ligase (i.e., treatment at 65°C for 15 minutes) as active DNA ligase will decrease packaging efficiencies.

Solutions:

Phage Dilution Buffer
10 mM Tris-HCl (pH 8.3)
100 mM NaCl
10 mM $MgCl_2$

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

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 OD_{600} = 0.8-1.0. Store the cells at 4°C until needed; cells may be stored for up to 72 hours.

Packaging Reactions:

1. Thaw the appropriate number of packaging extracts at room temperature. For every two packaging reactions, thaw one extract then place on ice.
2. When thawed, immediately transfer half (25 µl) of each packaging extract to a second 1.5-ml tube and place on ice.
3. Add the substrate DNA (10 µl [0.2 µg] of the control DNA) to a tube containing 25 µl of extract. If performing an odd number of packaging reactions, the remaining 25 µl of extract can be refrozen at –70°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 25 µl of thawed extract to each reaction tube at 30°C (If performing two packaging reactions, thaw another tube of extract and add 25 µl to each tube.) and incubate the reaction(s) for an additional 90 minutes at 30°C.
7. Add 500 µl of phage dilution buffer and mix by gentle vortexing.
8. Add 25 µl of chloroform and mix by gentle vortexing (store at 4°C).
9. Assay the packaged phage by titering on the appropriate bacterial strain (LE392MP for the control).

Titering Phage Extracts:

1. Make serial dilutions of the packaged phage in phage dilution buffer. Use 10^{-5} 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 (use LE392MP for the control reactions) and incubate for 15 minutes at 37°C.
3. Add 3.0 ml of melted supplemented (10 mM $MgSO_4$) LB top agar (cooled to ~48°C). Vortex gently and pour onto pre-warmed (37°C) LB plates. Allow the top agar to solidify and then incubate overnight at 37°C.
4. Count the plaques and determine the titer (pfu/ml) and packaging efficiency (See sample calculations).

Sample Calculations:

If there were 110 plaques on a 10^{-6} dilution plate, then the titer, pfu/ml, (where pfu represents plaque forming units) of this reaction would be:

$$\frac{\text{(\# of plaques) (dilution factor) (1000 µl/ml)}}{\text{(volume of phage plated [µl])}} \text{ OR } \frac{\text{(110 pfu) } (10^6) \text{ (1000 µl/ml)}}{\text{(100 µl)}} = 1.1 \times 10^9 \text{ pfu/ml}$$

The packaging efficiency (pfu/µg DNA) of this reaction would be:

$$\frac{\text{(\# of plaques) (dilution factor) (total reaction vol.)}}{\text{(vol. of dilution plated) (amount of DNA packaged)}} \text{ OR } \frac{\text{(110 pfu) } (10^6) \text{ (550 µl)}}{\text{(100 µl) (0.2 µg)}} = 3 \times 10^9 \text{ pfu/µg}$$

5. Related Products

The following products are also available:

Desc.	Concentration	Quantity
pWEB-TNC™ Cosmid Cloning Kit		
WEBC931		1 Kit
pWEB™ Cosmid Cloning Kit		
PC8805		1 Kit
EpiFOS™ Fosmid Library Production Kit		
FOS0901		1 Kit
Lambda Terminase		
LT4450	2 U/µl	50 Units
LT44200	2 U/µl	200 Units

6. 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.

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