

MaxPlax™ Lambda Packaging Extracts

Cat. Nos. MP5105, MP5110 and MP5120

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 $>1 \times 10^9$ pfu/ μ g 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*), (*λ imm434*, *clts*, *b2*, *red3*, *Dam15*, *Sam7*)/ λ]

** BHB2688: [N205 *recA*⁻, (*λ imm434* *clts*, *b2*, *red3*, *Eam4*, *Sam7*)/ λ]

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 $>1.0 \times 10^9$ pfu/ μ g of control λ DNA, when stored as directed for one year from the date of purchase.

Related Products: The following products are also available:

- pWEB-TNC™ Cosmid Cloning Kit
- pWEB™ Cosmid Cloning Kit
- EpiFOS™ Fosmid Library Production Kit
- Lambda Terminase

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.

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

–continued
Lit. #65

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 μ l 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 MgCl₂

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 MgSO₄) 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 MgSO₄ + 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.

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 μ l) of each packaging extract to a second 1.5 ml tube and place on ice.
3. Add the substrate DNA (10 μ l [0.2 μ g] of the control DNA) to a tube containing 25 μ l of extract. If performing an odd number of packaging reactions, the remaining 25 μ l 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 μ l of thawed extract to each reaction tube at 30°C (If performing two packaging reactions, thaw another tube of extract and add 25 μ l to each tube.) and incubate the reaction(s) for an additional 90 minutes at 30°C.
7. Add 500 μ l of phage dilution buffer and mix by gentle vortexing.
8. Add 25 μ l 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 MgSO₄) 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 below).

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:

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

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

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

MaxPlax™ Lambda Packaging Extracts Kit Contents

MaxPlax Lambda Packaging Extracts are available in 5, 10 and 20 extract sizes.

The 20 extract size kit contains the following reagents:

MaxPlax <i>in vitro</i> Lambda Packaging Extracts	20 tubes at 50 µl/tube
Ligated Lambda Control DNA (λcl857 <i>Sam7</i>) (1 µg @ 0.02 µg/µl)	50 µl
Control plating <i>E. coli</i> strain LE392MP, glycerol stock.....	250 µl
[F ⁻ e14 ⁻ (McrA ⁻) Δ(<i>mcrC-mrr</i>) (Tet ^R) <i>hsdR514 supE44 supF58 lacY1</i> or Δ(<i>lacIZY</i>)6 <i>galK2 galT22 metB1 trpR55 λ⁻</i>]	

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 CO₂-impermeable foil pouch.

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