Antibody Design LABORATORIES

CM13K Trypsin-Sensitive Helper Phage

INSTRUCTION MANUAL

CM13K Trypsin-Sensitive Helper Phage for Phage Display

Catalog #: PH050L, PH050P

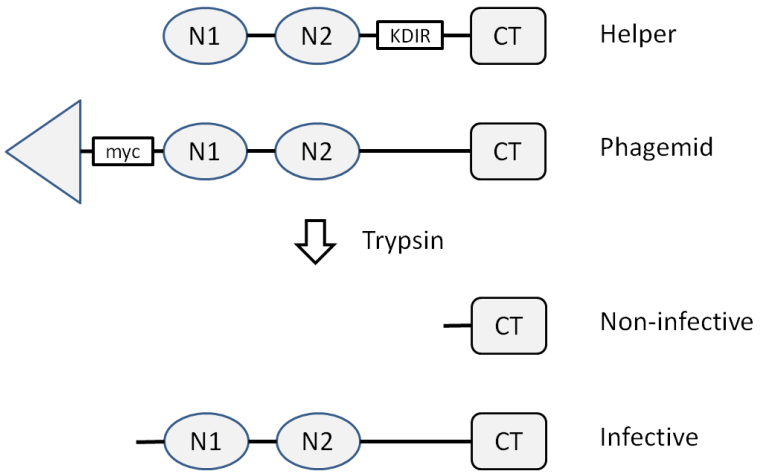
Version: A1.2 – January 2023

Table of Contents

Legal and Disclaimers

Description

CM13K is a helper phage especially engineered for phage display. CM13K is a derivative of CM13 (cat# PH020L) that contains a trypsin-sensitive linker between the N2 domain and the CT domain of the coat protein III. CM13K is therefore very similar to KM13 helper phage (1) and AGM13 helper phage (2). Upon exposure to trypsin, the N1N2 domains are excised and CM13K protein III becomes unable to mediate transduction, since the N1 and the N2 domains of protein III are required for efficient transduction of *E. coli*. Therefore, transduction after trypsin treatment will almost exclusively derive from fusion phage bearing a wild-type, trypsin-resistant protein III typically made by the phagemid (see illustration below). Moreover, it has been shown that trypsin release of bound phage is far superior to classical acidic elution (3). Together with the inactivation of the protein III deriving from the helper phage, the use of CM13K and trypsin elution results in more efficient and more selective biopannings.



CM13 is a derivative of M13KO7 (4) containing the interference-resistant ir3B A->G mutation (5) at position 8418 of M13KO7. This mutation is also found in VCSM13 helper phage. CM13- and CM13K-infected cells produce similar or higher levels of virions than M13KO7 while giving similar levels of display on the minor coat protein III. These helper phages make preparation of virions particularly efficient at small scale, thus facilitating the overall screening process. This preparation contains enough helper phage to superinfect up to 1 L (1000 ml) of TG1 culture. Other bacterial strains may require different amounts of CM13K helper phage.

CM13K virions were isolated from the supernatant of infected *E. coli* TG1 cells, purified by PEG precipitation and resuspended in 50% glycerol TBS buffered. The suspension is in liquid state at -20ºC.

Components

Product:	Purified CM13K Helper Phage
Catalog number:	PH050L, PH050P
Quantity:	1 ml (PHP050L) or 5 x 1 mL (PHP010P)
Titer:	2.0 x10^{12} pfu/ml

Storage conditions

Storage at -20ºC is recommended.

Related Helper Phage

HELPER PHAGE	CATALOG	DESCRIPTION
M13KO7 Helper Phage	PH010S	Original M13KO7 from Messing's lab
M13KO7 Helper Phage (concentrated)	PH010L	Concentrated M13KO7
M13KO7 Helper Phage (Pack 5 x 1 mL)	PH010P	Pack size (5 x 1 mL)
CM13 Interference-Resistant Helper Phage	PH020L	Variant of M13KO7 giving higher virion production
CM13 Helper Phage (Pack 5 x 1 mL)	PH020P	Pack size (5 x 1 mL)
M13KO7d3 pIII-Defective Helper Phage	PH030L	Discontinued helper
CM13d3 pIII-Defective Helper Phage	PH040L	pIII-Defective helper for multivalent display
CM13K Trypsin-Sensitive Helper Phage	PH050L	Trypsin-sensitive helper phage for improved elution and screening
CM13K Trypsin-Sensitive Helper Phage (Pack 5 x 1 mL)	PH050P	Pack size (5 x 1 mL)

Limited Product Warranty

This warranty limits our liability to the replacement of this product. No other warranties of any kind express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Antibody Design Labs. Antibody Design Labs shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

For research use only; not intended for any animal or human therapeutic or diagnostic use.

Protocol

Overview

The following guidelines are given for the production of virions displaying a polypeptide fused to the minor coat protein III of the M13 filamentous bacteriophage. For the production of single-stranded DNA using CM13K, please consult reference (6).

This protocol is limited to the step of transduction (or superinfection) by CM13K helper phage of phagemid-containing bacteria. For additional methods and protocols such as purifying virions, quantifying virions, measuring titers, and/or preparation of basic media & solutions please consult **Antibody Design Labs** online technical resources at http://www.abdesignlabs.com/technical-resources/.

CM13K helper phage is suitable for the infection of F^+ or F' *E. coli* bacteria containing a phagemid vector having an f1 or f1-like origin of replication. The protein III fusion is encoded by the phagemid vector while the helper phage brings all the necessary elements for efficient DNA packaging and assembly of virions.

The following protocol is given for the phagemid vector pADL-10b (**Antibody Design Labs** product number PD0105), an ampicillin-resistant phagemid with a full-length g3p fusion protein under the control of a *lac* promoter. Conditions are also optimized for single phage production in SS320 or TG1 *E. coli* strains in 2xYT medium and may required custom adjustments for different vectors, other strains, other growth media, and/or preparations of libraries.

Procedures

Working with filamentous phage

Keep the bench clean and regularly wiped with 2% bleach to limit phage cross-contamination and only use filtered tips to prevent aerosol contaminations. Phages are known to survive standard autoclaving conditions and are not removed by 0.22 µm filtration. Phages are either killed by heat-treating dry, autoclaved materials in an oven for 4 hours at 105ºC (7) or by incubation in 2% bleach for at least 1 hour. We recommend to extensively wash with hot water all glass and plastic-ware, then submerge (tubes) or incubate (flasks) with a 2% solution of bleach for at least one hour. Heat-resistant glassware can then be autoclaved in an autoclave that is never used for biological waste while sensitive plastic-ware can be used directly or at best heat-treated as described above.

Preparation of bacterial cells

1. Peak a single colony from a freshly streaked plate with the phagemid-containing bacteria and inoculate a 3-ml culture of 2xYT medium supplemented with ampicillin 100 µg/ml and glucose 1% w/v. In absence of IPTG and presence of glucose 1% w/v, the expression of the g3p fusion protein is repressed; this helps preventing loss of phagemid or selection of insertless clones, and improves library integrity.

2. Incubate o/n at 37ºC with agitation at 250 rpm.

3. In the morning, dilute an aliquot of the culture 1:20 v/v with fresh 2xYT medium in a new culture tube and incubate for one hour at 37ºC with agitation at 250 rpm. It is important to dilute the glucose to 0.1% or less to prevent the catabolite repression of the *lac* operator.

4. Take a small aliquot in sterile conditions and measure the absorbance at 600 nm of a 1:10 dilution in 2xYT medium (A_{600}). With SS320 or TG1 strains, absorbance should be between 0.4 and 0.5 OD and cells are ready to be transduced. If necessary adjust the length of incubation to your conditions.

Helper phage transduction

We recommend incubating directly at 37ºC with agitation at 250 rpm for 30 min to one hour after adding the helper phage. The total number of transductants is not increased by either pre-incubating bacteria without agitation for 10 min prior to adding the helper phage (sometimes said to regenerate pili destroyed by strong agitation) or after adding the phage (sometimes said to promote transduction). In fact we have noted that in our conditions a lack of agitation during the transduction period results in smaller number of transductants and variable virion production. We also recommend adding the helper phage when the bacterial culture reaches an optical density at 600 nm between 0.4 OD and 0.5 OD; large amounts of non-superinfected cells may decrease virion production above 0.5 OD while disparities caused by differences in phage growth rates will be amplified at lower ODs.

1. Optionally vortex vigorously CM13K and collect with a brief microcentrifugation; this treatment will disrupt potential phage aggregates and is indicated when reproducible and precise quantities of helper phage are desired.

2. Add 1 µl of CM13K phage per 1 ml of bacterial culture with A_{600} at 0.5 OD. This corresponds to a multiplicity of infection (MOI) around 14 and a number of pfu roughly 3 time to the number of bacteria in the culture medium.

3. Incubate for 1 h at 37°C and 250 rpm; some protocols only recommend 30 min incubation for this step.

4. Add ampicillin 100 µg/ml, kanamycin 50 µg/ml, and IPTG 200 µM; lower the temperature to 30ºC and incubate 8 h to overnight before proceeding to phage purification. The amount of IPTG to add depends on the phagemid, e.g. phagemids such as pADL-23c, pHEN2 or pComb3 do not require IPTG while pADL-10b does require IPTG.

Trypsin digestion & phage elution

We recommend using PBS for the last washes before elution as other buffers may alter trypsin specificity. Digestion of the trypsin-sensitive protein III can be achieved with trypsin-EDTA solutions typically used for cell culture (e.g. Trypsin 0.25% EDTA). Treatment at 37ºC for at least 15 min is recommended. For elution, the linker between the fusion protein and protein III must be trypsin-sensitive, either by engineering or by using a phagemid bearing a trypsin-sensitive tag such as a Myc tag (e.g. phagemid pADL-23c, cat# PD0111). Eluted phage can be stored several days at 4ºC or used directly for transduction.

Troubleshooting

The production of virions using helper phage is usually straightforward.

- Insure the presence of pili by applying selective conditions during the growth of bacterial stocks (streak TG1 bacteria on M9-minimal medium plates) or during the culture itself if maintenance of the F' episome is mediated by antibiotic resistance, e.g. tet^R phenotype for SS320 (add tetracycline 50 µM to the growth medium).
- Streaking a kanamycin-containing plate before adding CM13K may reveal contamination by another helper phage.
- Lack or low virion production when bacterial cultures are too dense at the time of transduction. This problem is amplified by immunity to superinfection induced by endogenous expression of full-length g3p fusion protein by the phagemid. Make sure to only use bacterial cultures with A_{600} equal or inferior to 0.5 OD when adding the helper phage, at best 0.4.
- Phagemid whose phage coat protein fusion expression is not properly regulated may lead to toxicity and arrest of cell division as soon as the glucose is removed from the culture medium. Therefore a culture not dense enough when the helper phage is added may produce very few virions. Always use freshly prepared bacteria and a culture to the recommend density value to maximize phage yields.

Appendix

MSDS Information

MSDSs (Material Safety Data Sheets) are available on **Antibody Design Labs** website at the corresponding product page.

Quality Control

Specifications are detailed on the online product page. **Antibody Design Labs** certifies that the product will perform according to these specifications.

Technical Support

Visit **Antibody Design Labs** website at **www.abdesignlabs.com** for technical resources, including manuals, vector maps and sequences, application notes, FAQs, etc.

FOR MORE INFORMATION OR TECHNICAL ASSISTANCE, CALL, WRITE, FAX, OR EMAIL US AT:

Antibody Design Labs
4901 Morena Blvd, Suite 203
San Diego, CA 92117

Email: support@abdesignlabs.com
Phone: 1-877-223-3104 (Toll Free)
(Monday – Friday 9:00 AM – 5:00 PM PST)

References

1. KRISTENSEN P, WINTER G. PROTEOLYTIC SELECTION FOR PROTEIN FOLDING USING FILAMENTOUS BACTERIOPHAGES. *FOLD DES*. 1998;3(5):321-328.
2. GUPTA A, SHRIVASTAVA N, GROVER P, ET AL. A NOVEL HELPER PHAGE ENABLING CONSTRUCTION OF GENOME-SCALE ORF-ENRICHED PHAGE DISPLAY LIBRARIES. *PLOS ONE*. 2013;8(9):E75212.
3. THOMAS WD, SMITH GP. THE CASE FOR TRYPSIN RELEASE OF AFFINITY-SELECTED PHAGES. *BIOTECHNIQUES*. 2010;49(3):651-654.
4. VIEIRA, J. AND MESSING, J. , EDITED BY R. WU AND L. GROSSMAN, *METHODS ENZYMOL.*, 153, PP. 3-11. SAN DIEGO: ACADEMIC PRESS (1987).
5. ENEA, V. AND ZINDER, N. , INTERFERENCE RESISTANT MUTANTS OF PHAGE F1, VIROLOGY 122, PP. 222-226 (1982).
6. SAMBROOK, J., FRITSCH, E.F., AND MANIATIS, T., IN MOLECULAR CLONING: A LABORATORY MANUAL. COLD SPRING HARBOR LABORATORY PRESS, NY, VOL. 1, 2, 3 (1989).
7. PHAGE DISPLAY: A LABORATORY MANUAL. EDITED BY C. F. BARBAS III, D. R. BURTON, J. K. SCOTT, AND G. J. SILVERMAN. COLD SPRING HARBOR, LABORATORY PRESS, COLD SPRING HARBOR, NY (2001).
8. SIDHU SS, LOWMAN HB, CUNNINGHAM BC, WELLS JA (2000). PHAGE DISPLAY FOR SELECTION OF NOVEL BINDING PEPTIDES. METHODS ENZYMOL.328:333-63.