Antibody Design LABORATORIES

M13KO7 Helper Phage

INSTRUCTION MANUAL

M13KO7 Helper phage for ssDNA production and phage display

Catalog #: PH010S, PH010L, PH010P

Version: A1.10 – January 2023

Table of Contents

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Description

M13KO7 is a helper phage designed for the production of single-stranded plasmid DNA for mutagenesis or sequencing (1) and for the production of virions for phage display. M13KO7 is an M13 phage that has the p15A origin of replication and the kanamycin-resistance gene from Tn 903 at the *AvaI* site at position 5825 of M13. M13KO7 does not contain the Met40Ile mutation in protein g2p as initially reported (1). Virions prepared with M13KO7 have a phagemid/helper ratio of around 9:1.

M13KO7 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 M13KO7 Helper Phage

Catalog number: PH010S, PH010L, PH010P,

Quantity: 1 ml (PHP010L, PH010S) or 5 x 1 mL (PHP010P)

Titer: 2 $x10^{12}$ pfu/ml (PH010L, PH010P) or 1.1 $x10^{11}$ pfu/ml (PH010S)

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)

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 g3p minor coat protein of the M13 filamentous bacteriophage. For the production of single-stranded DNA using M13KO7, please consult reference (2).

This protocol is limited to the step of transduction (or superinfection) by M13KO7 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/.

M13KO7 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 g3p fusion protein 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 the *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 preparation 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 (3) 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. Pick 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 close to 0.5 OD and cells are ready to be transduced. If necessary adjust the length of the 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 number of transductants increases over time, especially during the last 30 min of incubation. 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. For product PH010L (concentrated M13KO7), add 1 µl of M13KO7 phage per 1 ml of bacterial culture with A_{600} at 0.5 OD. This corresponds to a multiplicity of infection (MOI) around 18 and a number of pfu roughly 3 times the number of bacteria in the culture medium. For product PH010S, use 9 µl of M13KO7 phage per 1 ml of bacterial culture with A600 at 0.5 OD. This corresponds to a MOI around 9 and a number of pfu equivalent to 1.5 time number of bacteria in the culture medium.

2. Incubate for 1 h at 37°C and 250 rpm.

3. 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.

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 M13KO7 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.

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:

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References

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