Antibody Design LABORATORIES

Phage-Competent™ SS320 Cells

INSTRUCTION MANUAL

Phage-Competent SS320 Cells for Phage Display

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Description

Product Description

Most phage display procedures involve time-consuming counting and amplification of phage preparations using bacterial cultures. These cultures require careful preparations to generate reproducible results and prevent contamination. Phage-competent cells are concentrated stabilized bacterial cells designed to cut down time to prepare bacteria and bring reliability and reproducibility during the step of transduction. With phage-competent cells you can prepare plaques at any time of the day in less than 30 minutes with infectivity often better than with cells made from o/n precultures.

Phage-competent cells can undergo multiple freeze-thaw cycles without losing their titer and can be expanded without latency to generate large volumes of cells ready for transduction. Phage infectivity is usually equal or higher than infectivity measured on bacteria freshly prepared and transduction gives more reliable and reproducible numbers of cfu and pfu.

Components

PRODUCT: Phage competent SS320 cells
hsdR2 mcrA0 araD139 Δ(araA-leu)7697 ΔlacX74 galK16 galE15(GalS) λe14$^-$ rpsL150(Str^R) spoT1 thi F'[proAB+lacIqlacZΔM15 Tn10 (tet^r)]

CATALOG NUMBER: PC002

QUANTITY: 10x 0.5 ml

TITER: 20 OD_{600}/ml, >2E10 cfu/ml

DOUBLING TIME: 33 min at 37º and 250 rpm in 2xYT medium

Reagents, consumables, and equipment to be supplied by user

Reagents: 2xYT medium, 2xYT agar plates, varied antibiotics, TBS depending on the procedure.

Consumables: Tubes type Falcon 5 ml with snap-cap (BD Bioscienses 352058), sterile 5 ml pipettes, microfuge tubes, sterile tips with air barrier.

Equipment: Personal protection equipment (e.g., lab coat, gloves, goggles), Bunsen burner, manual pipettors, spreaders, a microcentrifuge.

Storage conditions

Store immediately at -80ºC in the original container.

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

Protocols

Procedures

Working with phage-competent cells

Always keep the cells at -80ºC in their original packaging. When needed, take out a tube, incubate at 37ºC in a water bath between 2 min and 5 min and immediately transfer on ice. When done, put the cells back in their original packaging at -80ºC. All testing were done with storage on ice no longer than 15 min and showed that cells could be thawed and frozen at least 5 times without losing their titer or their transducibility. Thawing the cells on ice will see a rapid decrease of the titer.

Procedure 1. Amplifying phagemid sub-libraries

Phage display biopanning usually operates by successive rounds of selection. At the end of one round, bound virions are eluted and further amplified for the next round. At the same time, the number of eluted virions is counted and single clones are either sequenced or assayed or both. A superinfection by a helper phage is required to generate the phagemid progeny.

Procedure

1. Thaw a tube of phage-competent SS320 cells at 37°C for 5 min and place immediately on ice. Dilute the cells to 0.5 $OD_{600\ nm}$ (1/40 dilution factor) with 2xYT medium to obtain the desired volume of cells; e.g. one tube of cells can make 20 ml at 0.5 $OD_{600\ nm}$.

2. If larger volumes of cells are needed, incubate the cells in a prewarmed 37ºC incubator at 250 rpm. Count a doubling of SS320 cell density every 33 min. For example, one tube of cells in 500 ml will reach 0.5 $OD_{600\ nm}$ after 2:20 h (500/20 = 25; log(25)/log(2) = 4.64; 4.64 x 33 min = 2:33 min). The first time cells are amplified, measure $OD_{600\ nm}$ at the end of the incubation and adjust the doubling time to your laboratory conditions.

3. Mix cells and phage eluate and incubate for 45 min at 37ºC and 250 rpm. The volume of the cells should always be equal or superior to the volume of the eluate. If the volume of the eluate is large, compensate the OD_{600} for the extra dilution factor; e.g. if you mix cells and eluate at equal volume, dilute the cells to 1.0 OD_{600} only.

4. Proceed to one 1/100 dilution (10 µl incubation + 990 µl 2xYT) followed by a tenfold serial dilution in 2xYT and spread 100 µl of each dilution onto a 2xYT agar plate supplemented with 100 µg/ml ampicillin and 1% w/v glucose (for most phagemid systems, adjust to your systems if other conditions are required) and incubate o/n at 37ºC in a dry incubator.

5. In the meantime, spread the remaining cells on large 2xYT agar plates supplemented with 100 µg/ml ampicillin and 1% w/v glucose (for most phagemid systems, adjust to your systems if other conditions are required) and incubate o/n at 37ºC in a dry incubator. Large 24 cm x 24 cm square plates can accommodate up to 1 ml; 150 mm diameter round Petri dishes can accommodate up to 0.5 ml; if the volume of cells is too large, proceed to a centrifugation at 3000 g for 10 min and resuspend cells in an appropriate volume of 2xYT medium.

6. The morning after, scrap the bacterial layer with a sterile glass spreader and between 5 ml and 10 ml of 2xYT medium per plate; collect all cells and put immediately on ice; measure OD_{600} after dilution in fresh 2xYT medium;

use preferably a spectrophotometer that accommodates wide 1-cm path cuvettes and dilute cells so that the optical density is no more than 0.5 OD_{600} or even lower; start with a 1/100 dilution factor.

7. Mix sterile glycerol 50% v/v in water with part of the cells to a final glycerol concentration of 10%; aliquot and store at -80ºC.
8. Dilute cells in 20 ml of 2xYT medium to 0.4 $OD_{600\ nm}$ and proceed immediately to the helper superinfection (see M13KO7 or CM13 manuals on Antibody Design Labs website).
9. In parallel, count the colonies on the dilution plates; correct for dilution and initial volume of the eluate and estimate the total number of eluted cfu. Store the dilution plates with well-individualized colonies at 4ºC for up to 2 days.
10. The day after, prepare virions from the amplified sublibrary as usual.

Notes

- Some old protocols suggest letting the cells rest at 37ºC for 10 min before transduction to allow pili to regrow. In our conditions (250 rpm, flasks without baffles) this step can be omitted.
- Many protocols suggest a resting period on the bench or at 37ºC without agitation after mixing cells with phage to enhance transduction. We found transduction more consistent and more efficient when bacteria and phage are agitated at 250 rpm and 37ºC immediately. This step should be omitted.
- Use the entire eluate for the first round to rescue every selected clone and only part of it the following rounds to limit counts that are usually higher. If necessary keep the remaining eluate (stable for weeks at 4ºC, months at -80ºC) for later use.
- Always analyze clones from the eluate, i.e. clones found on the dilution plates, rather than clones from the amplified sublibrary; diversity will be higher. If you need to analyze more clones, infect cells with some of the leftover eluate.

Procedure 2. Counting colonies with fADL™ phage vector

fADL-1 is a phage vector sold under product number PD0010. fADL-1 is a derivative of the vector fd-tet developed by George Smith for phage display (see fADL-1 manual for further information). fADL1 confers kanamycin resistance to the bacterial host and produces colonies on agar plate. fADL-1 phages are preferably produced on SS320 bacterial strain.

1. Dilute the phage preparation in either TBS or 2xYT medium. The dilution factor can be estimated for PEG-precipitated virion preparations by converting the spectrophotometric absorption values into virion concentrations (see ADL web site) and estimating the infectivity to ~5% (virions/ml x infectivity ~= cfu/ml). For accurate counts, the MOI (multiplicity of infection or virion/bacteria) should be less than 0.1.
2. Thaw a tube of phage-competent SS320 cells at 37°C for 5 min and place immediately on ice.
3. In a 5 ml Falcon tube mix 5 µl of phage dilution, 5 µl of cells and 190 µl of 2xYT medium. Include in each series a negative control without phage. Put the cells back at -80°C in their original packaging.
4. Incubate for 45 min (30 min to 1 h) at 37°C and 250 rpm and spread 100 µl in duplicate on a 2xYT agar plate supplemented with 50 µg/ml of kanamycin.
5. Incubate o/n at 37°C and count the colonies.

Procedure 3. Counting colonies with fUSE5 derivative phage vectors.

The procedure is identical to Procedure 1 except that 0.2 µg/ml tetracycline is added during the incubation; this low concentration of tetracycline induces expression of the tetracycline resistance gene without inhibiting protein synthesis. Supplement agar plates with 40 µg/ml tetracycline instead of kanamycin.

Procedure 4. Counting colonies by the spot technique

For all fd-tet derived phage vectors (Procedures 2 & 3), transduced cells can be spotted (20 µl/spot) on agar plates supplemented with the proper antibiotic; plates dried o/n in a 37°C incubator should be used. Around 20 spots can fit on a regular 100-mm plate and multiple samples can be counted on a single plate. Similarly, serial dilutions in 2xYT of infected bacteria can be spotted with a multichannel pipette over 2 plates. If using serial dilution, calculate the mean cfu over three successive spots adjusted for the dilution factor.

Troubleshooting

- **Titers/cell counts are lower than expected**: Never thaw cells on ice or keep them at room temperature for prolonged period of time; always put the tube back in its original packaging as soon as possible. In such a case, discard the tube and open a new one.
- **Plaques or colonies are found in the control**: The tube is contaminated; discard the tube and open a new one. Note that selection by kanamycin or ampicillin may give satellite colonies when large quantities of cells are spread. If the situation becomes chronic, the level of contamination is likely too high in your laboratory. Clean all benches and wipe all instruments with bleach 10%; clean all pipettors and UV them o/n in an enclosed hood and only use aerosol filter tips. Once the source of contamination has been eliminated, maintain good working habits in the lab.

Recipes

2xYT MEDIUM

Use a commercial preparation. Dilute 31 g/l of deionized water and autoclave.

AMPICILLIN 100 mg/ml STOCK SOLUTION

Dissolve ampicillin sodium salt to 100 mg/ml in pure water and filter-sterilize through a 0.22 µM filter.

GLUCOSE 20% w/v

Dissolve 20 g of dextrose (D-glucose) in 80 ml of pure water; adjust volume to 100 ml with water and filter-sterilize through a 0.22 µM filter.

TBS 10x

Tris Buffer Saline 10X, of commercial origin to prevent bacterial/protease contaminations. Use 1x after dilution in pure water.

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.

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.

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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