Special Instructions

Supply, Storage, Propagation and Purification of Phages

Supply and Storage of Phages

New: the DSMZ leaves it to the customer in what form a phage will be ordered, either as liquid suspension ("active culture") or filter-dried (in double-vial glass ampoule), see our FAQ on the phage homepage at http://www.dsmz.de/catalogues/catalogue-microorganisms/groups-of-organisms-and-their-applications/phages/faq.html

Active culture:
DSMZ phages are delivered as 1 mL portions of bacteria-free lysates in the host's growth medium if an "active culture" of a phage was ordered. Bacterial cells and debris have been eliminated by centrifugation and subsequent membrane filtration. All lots of phage stocks are tested for phage titre and plaque morphology/plaque purity. Phage suspensions delivered to our customers are usually high titre solutions in the range from 1×10^8 - 1×10^{11} pfu/mL (pfu = **p**laque **f**orming **u**nits/mL). Phages should be stored cool immediately upon receipt. Don't freeze phage suspensions without adding a cryoprotectant. When stored cool, most of the phages will remain active without significant activity loss for some months. However, the DSMZ does not guarantee for phage survival over longer storage periods, please see our homepage information. Phage lysates can be stored deeply frozen or in liquid nitrogen for long-term purposes if a cryoprotectant has been added e.g., 10% (v/v) of sterile glycerol, final concentration.

Filter-dried samples:
Due to better survival during transport over long distances (e.g., transcontinental) or if the customer likes to store the phage for a longer period before experiments begin, the DSMZ recommends to order filter-dried phages.
The customer will receive the phage dried on filter paper that has been vacuum-dried in the double-vial glass ampoule typical for DSMZ cultures. Please, observe our separate hints for opening these ampoules and for further propagating the phages.
http://www.dsmz.de/catalogues/catalogue-microorganisms/groups-of-organisms-and-their-applications/phages/phages-handling-of-freeze-dried-ampoules.html

Generally:
According to our **Terms and Conditions**, all kinds of biological material supplied by the DSMZ is for immediate use in the authorised laboratory only. All our phages are tested by using the host strain that we recommend. Therefore, please observe our **FAQ** on the phage homepage and the specific information on each individual phage.

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Propagation of Phages

Please, use only the recommended bacterial host strain for a particular phage.

The DSMZ will not guarantee for successful phage propagation when other hosts are used, independently of the host spectrum of a phage. New high titre phage stocks can be prepared directly by phage propagating and "titering-up", by starting from single plaques or from phage suspensions or by floating agar plates that have confluent lysis: always use a suitable phage buffer. Performing lysis is possible in liquid bacterial host cultures or on plates by using the double agar layer technique with top and bottom agar. **Lysis on plates**: The bottom agar is the normal carrier layer with usual agar concentration and nutrients for the host; the top layer has half of the agar concentration and contains the host bacteria mixed in this "soft agar" so that a homogenous thin layer of bacterial lawn develops. This is suitable for adding the phages either as drops / spots or the phages may be added already to the top layer so that single plaques will develop and will deliver an optically ideal picture to evaluate the purity of a phage or to count the plaques for estimating the titre. When lysis on plates is performed, the plates can be incubated by shaking them carefully on a plate shaker for few hours (host bacterium and phage are incubated together), high titres can be received. The soft agar layer with confluent lysis can then be scraped off. Centrifugation for removing agar and cell debris and filtration through 0.45 µm membrane filters will follow. **Liquid lysis**: the bacterial culture growing together with added phages should be observed because of possible phage-resistant bacterial cells overgrowing the culture. Lysis may be quick or require a longer time. Also, the culture must be carefully shaken because of phage adsorption. Lysis on plates may lead to better phage titres than in liquid lysis due to this agitation-adsorption problem. When the culture looks more or less clear, bacterial debris should be sedimented by centrifugation followed by filtration through a 0.45 µm membrane filter.

Single plaque isolation: Upon severe loss of titre, a new phage suspension can be prepared from one single plaque by recovering the plaque with e.g. an Eppendorf tip and subsequent suspension in a drop of phage buffer. This can be applied to a bacterial host culture or spread on the surface of a host agar plate (see above, lysis on plates). By applying subsequent steps of culturing this plaque with bigger volumes of the bacterial host, "titering-up" is possible. At the same time, this is a phage purification procedure because it started with a single plaque. High-titre phage stocks can be made in this way.

Purification of Phages

In general, the microbiological principle is comparable to that of purifying bacteria: phages can be purified by isolating single plaques whereas bacteria are purified by isolating single colonies because both are the result of one single phage or one single cell, respectively. Even more, phages can be streaked on agar plates like bacteria (using the double layer agar technique, see above): for this purpose, it is recommended to use disposable plastic loops with a small loop; it is easy to streak a plaque according to the streaking principle for bacteria. New single plaques can be received when this technique is applied. In case of contaminated phage suspensions, subsequent streaking will lead to a real and simple purification of a phage. A phage always needs its host for "growing". "Growing" means successful infection of the host (propagation of phages, see above). Phage and host must find optimal conditions for phage adsorption and the complete infection cycle. The yield of phages depends on many factors e.g., the burst size, the moi (multiplicity of infection, the host/phage ratio) etc. Phages may be purified for getting laboratory stock solutions or for further applications of the phages like transmission electron microscopy (TEM). For TEM, phage solutions must be reasonable pure, without debris. For this purpose, it is optimal to perform cesium chloride density gradients to get a single band containing the phage. Also, for isolating DNA of the phage, a cesium chloride density gradient is recommended. Further details are not given here. Also, phages for any therapeutic use require special standardised purification steps that are not given here.

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A widely used Medium and buffer for phage propagation:

General Growth Medium

Difco Nutrient Broth	8 g
NaCl	0.8 g
Difco Bacto Agar	15 g
Distilled Water to	1.0 L

Phage Buffer

Na_2HPO_4 anhydrous	7 g
KH_2PO_4 anhydrous	3 g
NaCl	5 g
0.1 M $MgSO_4$	10 mL
0.1 M $CaCl_2$	10 mL
H_2O to	1.0 L

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DSMZ, August 2014