Leibniz-Institut
DSMZ-Deutsche Sammlung von
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Supply, storage, propagation and purification of phages

Supply and storage of phages

If a phage is preserved and available in both forms, 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 phage FAQ

https://www.dsmz.de/collection/catalogue/microorganisms/phages-faq

Phage suspensions: Our 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 or if the phage is only available in this form. Bacterial cells and debris have been eliminated after lysis by centrifugation and subsequent filtration through single-use cellulose acetate syringe filters, 0.2 or 0.45 µm pore size. All lots of phage stocks are tested for titer and plaque morphology/plaque purity. Phage suspensions delivered to our customers are ready-to-use for propagation in the recipient's laboratory and have usually titers ranging from 1×10^8 - 1×10^{11} pfu/mL (pfu = **p**laque **f**orming **u**nits). We do not provide phage titer data as titers may drop during the test intervals in our laboratory. Phages should be stored cool and dark 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 frozen for long-term purposes if a cryoprotectant has been added e.g., 10% (v/v) of sterile glycerol, final concentration.

Filter-dried phages: For better survival during transport over long distances (e.g., transcontinental), the DSMZ recommends to order filter-dried phages. Very few phages known to be unstable in suspension can only be delivered that way to guarantee intact and infectious particles. The customer will receive the phage dried on filter paper that has been vacuum-dried in double-vial glass ampoules typical for DSMZ microorganism cultures. Please, observe our separate hints for opening these ampoules:

https://www.dsmz.de/collection/catalogue/microorganisms/culture-technology

and for further propagating the phages:

https://www.dsmz.de/collection/catalogue/microorganisms/special-groups-of-organisms/phages

Phage suspensions have advantages over filter-dried phages as they are ready-to-use for starting an initial phage propagation and more flexible regarding the method of propagation (e.g., spotting on host plates or directly infecting a host culture). Also, phages may be streaked on host plates to receive single plaques. Before starting to work with the pages the titer has to be determined in the recipient's laboratory!

General: According to our **Terms and Conditions**, all biological materials supplied by the DSMZ are for immediate use in the authorized laboratory only and exclusively for laboratory use, not for direct application in humans or animals. Our phages are tested by using the host strain that we recommend. Therefore, please observe our phages-**FAQ** and the specific catalogue information on each individual phage:

https://www.dsmz.de/collection/catalogue/microorganisms

Propagation of phages

Please, use only the recommended bacterial host strain for each phage. You will find the recommended host strain and its DSM number in the catalogue entry of each 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 titer 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: use a suitable phage buffer, e.g. buffer mentioned below. Performing lysis is possible in liquid bacterial host cultures or on plates by using the double agar layer technique with top and bottom agar. Please, have a look at the recommendation on propagation given for each individual phage in the catalogue.

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. Depending on the phage plaque sizes or other factors, the top layer agar concentration may be adapted from 0.3% to 0.75%. This technique is suitable for adding phages either as drops / spots on the top layer or the phages may be added already into the soft agar so that single plaques will develop and deliver an optically ideal picture to evaluate the purity of a phage or to count plaques for titer estimation. When lysis on plates is performed, the plates can be incubated by shaking carefully on a plate shaker for few hours (host bacterium and phage are incubated together), high titers 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 filters will follow and, if required, a second filtration through 0.2 µm filters.

Liquid lysis: The bacterial culture growing together with added phages should be observed until lysis occurs because of possible phage-resistant bacterial cells overgrowing the culture. Lysis may be quick or require a longer time, depending on many factors, especially on the individual phage-host system and the lysate may get completely clear or remain turbid. Also, the culture must be carefully shaken because of phage adsorption, don't use baffled Erlenmeyer flasks. Lysis on plates may lead to better phage titers than in liquid lysis. If liquid lysis does not result in sufficient titers, lysis on plates may be more successful.

Single plaque isolation: 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 small volume (few mL) bacterial culture or spread on the surface of a host agar plate (see above, lysis on plates). By performing subsequent steps of culturing with increasing volumes of the bacterial host, "titering-up" is possible. High-titer phage stocks can be made in this way.

The phage host: The choice of the correct phage host strain is crucial for phage propagation. Furthermore, the ideal MOI, multiplicity of infection (ratio of phage particle number to host cell number), should be considered for each phage-host system in order to achieve optimal lysis and high phage titers: active phage particle numbers of phage suspensions are known by the phage titer (pfu/mL), cell numbers of cultures are counted by using counting chambers or estimated by determining the $OD_{600\ nm}$ before phage infection. There is no ideal MOI for all phages, it is phage- or phage-host-specific. Often, the best MOI is 0.1, 0.01 or less. Due to the lysis dynamics, low MOI often results in higher titers. But, this cannot be generalised. The DSMZ does neither provide MOI data nor host spectra. For the host, optimal growth conditions must be used and the culture must be freshly inoculated from a fresh agar plate or pre-culture.

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For phage infection, most phages require fresh log phase cultures with low $OD_{600\ nm}$ (often 0.2 – 0.3), the $OD_{600\ nm}$ of the culture must be observed before infection and the culture, if overgrown, must not be diluted to get the desirable low $OD_{600\ nm}$ as the cell status and phage receptor expression are controlling phage adsorption. Host agar plates should generally be fresh, plates for all phage work must be freshly poured. Agar plates from industrial providers are often too dry, dry plates are a titer-limiting factor. Titers of the phages have to be determined before phage propagation!

Microbiological phage purification on laboratory batch level

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, both originate from one single phage or one single cell, respectively. 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; 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 or, testing if a phage is lytic on a new host is simple and fast. In case of contaminated phage suspensions, subsequent streaking will purify a phage. A phage always needs its host for “growing” which 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, host/phage ratio) etc. Phages may be purified for getting laboratory stock solutions or for further applications or transmission electron microscopy (TEM) visualization. For TEM, phage solutions must be reasonably pure, without debris. For this, it is optimal to perform cesium chloride density gradients to get a single band containing the phage. Also, for isolating phage DNA, a cesium chloride density gradient is recommended. Further details are not given here. It is not the purpose of this brief document to include information on further purification procedures prior to phage application.

Widely used SM Buffer for phages:

0.1 M NaCl
8 mM MgSO4
50 mM Tris-HCl
adjust to pH 7.5

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