

LentiKats®

Tips & Tricks



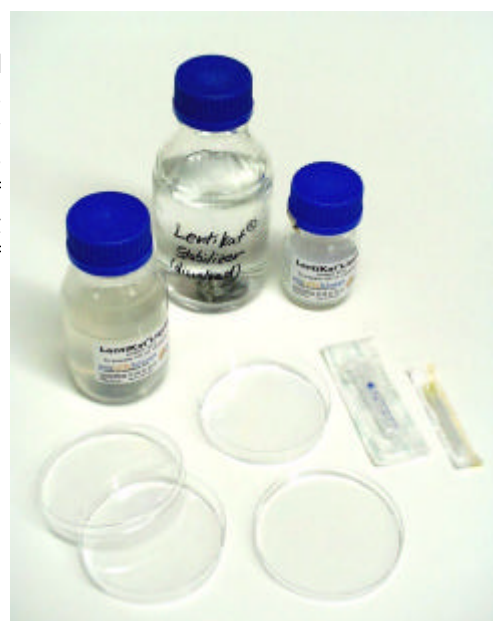
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<http://www.geniaLab.de/download/tt-english.pdf>

1 LentiKats® in your lab

LentiKat®Liquid is shipped sterile in a ready-to-use form. The bottle contains 80 g (200 g) of specially designed PVA-solution with various additives. Its density is approximately 1 g/mL. The composition is designed for entrapment of 20 mL (50 mL) biocatalyst suspension - giving a maximum of 100 mL (250 mL) of LentiKats®. If you want to entrap less material, e.g. 4 mL of biocatalyst suspension, you have to use of course only 16 g of LentiKat®Liquid.

Please read the complete manual carefully before starting and be sure to have all necessary equipment ready at hand!



2 How to produce LentiKats®

Prepare LentiKat®Stabilizer with distilled water from the included powder and set aside for later use. The solution may be sterilized by autoclaving for 20 min at 121°C (250 F).

The amount of stabilizer you will need strongly depends on the amount of LentiKats® to be prepared and the equipment you will use. As a rule of thumb you can calculate a need of 200 mL for 5 g and 1000 mL for 50 g.

Melt LentiKat®Liquid by placing in a water-bath at 95°C (200 F). Use a magnetic stirrer for mixing thoroughly. Melting is accomplished when the liquid is homogenous and clear like water.

A microwave oven might be used at this step but you have to be careful not to overheat the bottle. The liquid is not altered by boiling, however, this implies danger to the people working.

DANGER! Do not open bottle with overheated LentiKat® Liquid!

To keep sterile conditions and, more important, to avoid loss of water you should not open the bottle during the melting process.

Afterward completely melting LentiKat® Liquid it is cooled down to an ambient temperature that is of no harm to your biocatalyst. You have to take into account that an undesired gelation process will occur at lower temperatures. However, at a temperature of 25°C to 30°C (77 F to 86 F) LentiKat® Liquid will be workable for several minutes.

After cooling the biocatalyst suspension is added and dispersed homogenously by mixing with a magnetic stirrer or vigorous shaking. If you want to entrap biomass wet matter, e.g. cells separated by centrifugation, you have to dilute it to a maximum of 50% biomass wet matter before adding to LentiKat® Liquid. Keep in mind to add the necessary volume as explained above.

For production of the LentiKats® a smooth plate is needed preferably of polystyrene. For example single-use petri-dishes give good results. Some materials, e.g. glass are not suitable at all. To optimize the following necessary partial drying it is advisable to use plates with a rim as low as possible. For petri-dishes this means to use the cover-lid instead of the bottom part!

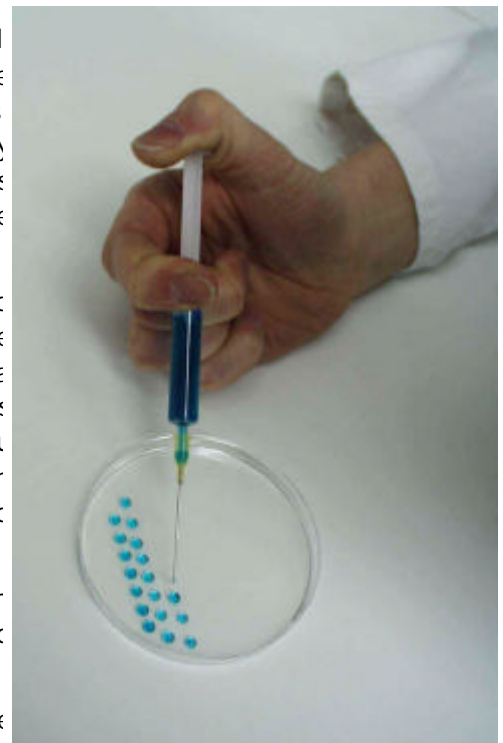
Each plate you are going to use has to be marked individually, to be weighed and the tare to be noted before going on. Using a standard syringe with a cannula (approx. 1.0 mm in diameter) you have to form droplets and drip them neatly onto the surface of the plate. You should try to form the droplets (approx. 3 mm in diameter and mass of 5 mg) as uniform as possible and also work as fast as possible.

After finishing a complete plate weigh it again immediately to determine the amount of LentiKat® Liquid on this plate.

The slower you are in making the droplets, the more unequal they dry. To start for the drying process for several plates simultaneously a finished plate can be covered. In case of using petri-dishes this can be done with bottom part of the dish.

The figures for the plates are best taken down in a table of the following format. A template for copying is found at the end of this manual:

<i>plate</i>	<i>empty plate</i>	<i>plate plus wet droplets</i>	<i>wet droplets (calculated)</i>	<i>desired weight (28% of wet)</i>	<i>desired weight for plate</i>
1	10,18	12,52	2,34	0,66	10,84
2	10,25	12,85	2,60	0,73	10,98
3	10,09	12,51	2,45	0,69	10,78



After weighing the plate the droplets have to be dried for the gelation. Drying can be done by leaving the plate exposed to air. To expedite removal of water a ventilator or fan for aeration from above may be useful. Maximum temperature during gelation should not exceed approx. 35°C (95 F) and depends on the biocatalyst that is entrapped.

Gelation of LentiKats® is complete if 72 % of the mass of LentiKat®Liquid that has been dripped on the plate has evaporated. Hence it is advisable to control weight of the plates regularly during gelation. Time for gelation strongly depends on drying facility used and the humidity.

If your biocatalyst is not sensitive towards drying you may carry on drying to remove almost all the water. This will result in extremely stable LentiKats®. However, the final size of LentiKats® and diffusion properties of the hydrogel will be influenced by this and have to be checked carefully.

When gelation is complete LentiKat®Stabilizer is poured on the LentiKats® for sloughing the gel particles and for re-swelling. LentiKats® will re-swell to about their original size by this treatment. After 2 or 3 minutes of contact with the stabilizer solution LentiKats® are easily to be removed from the surface and to be put into a bottle containing a 10-fold surplus of LentiKat®Stabilizer.

When petri-dishes are used for the preparation of LentiKats® the contact of stabilizer solution with these LentiKats® is easily done by pouring it over them. After re-swelling the same solution can be removed and be used for the next petri-dish to save LentiKat®Stabilizer solution.

LentiKats® have to be stirred for at least 2 hours in stabilizer solution to give a maximum of mechanical stability for the later application. Any shortening in the times of operation given above have to be proofed for each case regarding their influence on stability of LentiKats® and are off our responsibility.

If your biocatalyst probably is sensitive towards a prolonged period of lack of medium components you can try to dissolve LentiKat®Stabilizer in your normally used medium to give a stabilizer solution better tolerated by the biocatalyst.

After stabilization is finished the supernatant has to be removed and can be replaced by the medium or solution you want to use your immobilized biocatalyst with. If foaming occurs it may be necessary to remove the supernatant again after a certain time of stirring. This effect will depend on the relation of LentiKats® and medium solution you will work with.

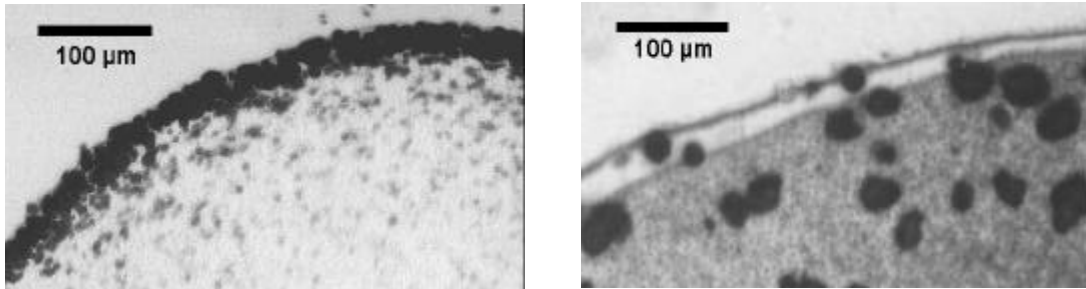
3 A few words about initial biomass content

If you are entrapping living cells which should form colonies inside the LentiKats® after immobilization the initial biomass content obviously influences the maximum specific activity, i.e. the product formation per h and mL of gel, you will find after growing to a steady state.

If initial biomass content is too high you will get kind of a shell-catalyst characterized by intensive biomass growth at the surface of LentiKats® leading to very poor nutrient supply in the central part of the gel and bad specific activity (see left picture below for example). If initial biomass content is too low the gel volume of LentiKats® is not utilized sufficiently. Hence optimum for initial biomass content has to be found for each application.

As a rule of thumb an initial biomass content of about 10^7 cells per mL of gel should be near optimum. This value was found for different bacteria. Cell number is more useful than weight of biomass since cells of different strains may strongly differ in their size - but one colony will have its origin in one cell not in a certain small portion of biomass.

If biomass content of LentiKats® is too high a rolling of LentiKats® may occur after some prolonged time of incubation. Besides decreasing biomass content there may be some relief by enlarging thickness of LentiKats® or enhancing hydrogel stability (duration of stabilization or further drying if possible).



If dead or resting cells are to be entrapped we recommend a biomass content of 10 % (w/w) biomass wet matter. If intracellular enzymes in more or less perforated cells ought to be used crosslinking the cell contents with glutardialdehyde might be helpful to prevent leakage of biocatalyst.

4 Working with LentiKats®

- Correctly stabilized LentiKats® tolerate a maximum temperature up to 50°C to 55°C (122 F to 131 F).
- pH values between 3.1 and 8.5 were tested for several days or weeks without any signs of disintegration of LentiKats®. Values beyond these values are strongly expected to be possible.
- If LentiKats® should be applied at some unusual conditions it is strongly recommended to do some experiments regarding long-term stability in advance.

5 Bits and pieces

Microorganisms entrapped in LentiKats® can be controlled microscopically. To enhance contrast of cells and hydrogel staining may be useful: treatment with 1:100 diluted Carbol-Fuchsin solution (ZIEHL and NEELSEN) gave good results after staining and decolourizing of 30 minutes each.

Working with your biocatalyst entrapped in LentiKats® probably you do not have to care as much for maintenance of sterile conditions. Contaminating cells will not be able to enter the hydrogel and replace the desired biocatalyst which hence will be in great surplus compared to any contaminating cells.

6 Safety Considerations

LentiKat®Liquid is for laboratory use only. The usual measurements when working with chemicals have to be applied. In case of accidental contact with skin or eyes rinse thoroughly with luke-warm water.

7 ... some advertising

For easier production of uniformly shaped LentiKats® a specially designed printer is available. This device transfers more than 400 identical droplets in one step. If your initial tests give good results and you intend to keep working with LentiKats® you should have a second thought about obtaining your own printer.

However, there is no need to buy the "pig in a poke". Depending on our stock you can also lease a printer!

If there are any questions left or comments to this manual please contact geniaLab® GmbH by eMail at info@geniaLab.com



LentiKat® experimental record

Date: _____

Description: _____

No.	empty plate	plate with wet droplets	wet droplets (calculated)	desired weight (____% wet dropl.)	desired weight for plate
1					
2					
3					
4					
5					
6					
7					
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