## **ADJUVANTS - FREQUENTLY ASKED QUESTIONS**

	QUESTION	ANSWER
Antigen mixture	How is the antigen-adjuvant mixture prepared?	Add the antigen solution with the adjuvant in a volume ratio of 1:1 to a sterile reagent vial and mix by shaking. Avoid foam formation. Use always a separate sterile needle for each animal.
	What is the optimum pH for injection?	GERBU Adjuvants have a pH range of 5.6 - 5.8. The recommended pH for injection is 7.0-7.3.
	Which are the recommended buffers for use?	We recommend Tris as a buffer in a range from 10 mM to 25 mM (pH = $7.3$ ). The exact concentration depends on the protein (antigenic properties).
	According to the manual, polyvalent buffers (phosphate / citrate) should be avoided.  Will the adjuvant flocculate?	If possible, use water (demineralized) to dilute the antigens. Polyanionic compounds can lead to precipitation. If unavoidable, use as small amounts of PBS as possible. Before injection, please assure that the antigen/adjuvant mixture did not coagulate.
	My antigen is sparely soluble. Which solvent do you recommend?	Ethanol. Dissolve the water-insoluble antigen in ethanol. Then slowly dilute this ethanolic pre-solution with a suitable aqueous buffer system to 10 times the volume and then prepare a 1: 1 mixture with the adjuvant. Please do not exceed the final concentration of 5% ethanol.
	Which additives and solvents should NOT be used?	We strongly advise not to inject denaturants (e.g. SDS, urea) and chelating agents (e.g. EDTA), as well as large-molecular buffer substances (e.g. HEPES).
Antigen pecularities	Are non-covalent bonds of protein complexes destroyed?	The quaternary structure of proteins is not affected. In this way, the functionality can be retained with the corresponding presentation of specific epitopes.
	Does GERBU Adjuvant mix with cells or DNA as Antigen?	You can mix GERBU Adjuvants with DNA or any plasmide.
		BSA acts as a small molecule carrier to enhance their antigenicity and presentation of the small molecule epitopes. As a clear, administrable mixture, dissolve 500 mg powdered BSA in 0.5 ml water (50% w / v) or 350 mg BSA in 0.65 ml 0.85% NaCl (35% w / v). For the immunization process, these solutions are mixed in a volume ratio of 1: 1 with GERBU Adjuvants directly beforehand.
	What can i do to improve the results for antigens with weak antigenicity?	Weak antigens need more efficient immune starters than strong antigens. However, the antigen dose used in the immunization should not be too high, to avoid immune tolerance of the host animal to the antigen. More frequent booster injections are recommended.
	Are there any information about the protein-binding capacity?	For this specific issue, please contact technical support.
Injection	How is the immunization process performed?	Bring the immunization suspension to room or body temperature (between 20 °C and 37 °C). Make sure it does not contain any large particles. By using sterile injection equipment, apply the doses into each immunization site slowly. The application is implemented subcutaneously into the subdermal fatty tissue. If possible, form a skin fold on the individual in order to apply the injection there. Please follow the corresponding instructions for use regarding the number of injection sites and the respective maximum volume.
	What dose can be given to young animals?	Compare weight to other animals according to the protocol.
	Is it also possible to inject intramuscularly?	Intramuscular application is usually well tolerated, but we do not recommend it as it causes more stress to the animal. Chicken may stop laying eggs.
	Why is GERBU Adjuvant not used intravenously?	Intravenous use violates the animal welfare act. It does not increase the overall efficiency.
	Are there any immunization reactions at the injection site?	The vaccine complex may act with a delayed effect, and short-term immunogenic reactions are possible. The adjuvant builds a depot and remains visible under the skin for a short time.





## **ADJUVANTS - FREQUENTLY ASKED QUESTIONS**

QUESTION ANSWER

GERBU Adjuvant was stored in the refrigerator (4-8 °C) and is unopened in the original packaging. There in, fine precipitations and agglutinations are visible and it appears viscous. Can the product still be used for injection?

GERBU Adjuvants are stable for 3 years from the date of manufacturing (at  $2-8\,^{\circ}$  C). If GERBU Adjuvants are stored without agitation in a cool place for an extended period of time, components may separate and crystallize. This has no impact on the quality of the product. As soon as being brought to room temperature and mixed well, a homogeneous suspension is obtained again.

How long is GERBU Adjuvants shelf life AFTER the first collection?

At least 1/2 year under sterile conditions in the refrigerator.

The adjuvant-antigen mixture is foamy / precipitated / clumpy. Can it still be used?

No, because the desired antigenicity is no longer given. Additionally, the animals might be harmed.

After GERBU Adjuvant has been mixed with the antigen, how long can this emulsion be kept under which conditions?

When an adjuvant is mixed with an antigen, the emulsion should be injected directly. If the antigen is stable, the emulsion can be stored for 3 months at room temperature. The stability of the antigen/adjuvant mixture is dependent on the properties of the antigen and the buffer.

Is the handling of GERBU Adjuvants comparable to Freund's Adjuvant (FCA/FIA)?

The preparation of the emulsion with two syringes is not necessary with GERBU Adjuvants. The antigen / adjuvant mixing ratio is 1: 1 in both cases. Please refer to the protocal for detailed instructions.

Are there differences in the individual lot numbers of the GERBU Adjuvants of a variety? That is, do the different lots have a different rate of generating antibodies in vivo? The manufacturing process is standardized according to GMP. All GERBU Adjuvants batch numbers are always subjected to internal quality control. It is examined if the specific parameters of the adjuvants lie within the specified range. The generation of specific antibody titers does not depend on particular batch numbers of GERBU Adjuvants. The generation of antibodies always depends on the the constitution of the animals and the properties of the antigen used.

I was using GERBU Adjuvant 10/100 / LQ before. Why is it no longer available?

The recipes at that time differed only slightly and mainly in terms of packing size. We have further improved the formula and combined the three products into one. Today it is called Adjuvant P.

I used a different adjuvant before. Now I want to switch to GERBU. Can I simply continue to administer GERBU adjuvant to the animals?

The use of different adjuvant systems can affect the results and the condition of the animals in unexpected ways. We recommend suspend of injections for an appropriate period of time before changing the adjuvant to give the animal's immune system time for regeneration. Within a short-term experiment, however, the adjuvant should not be changed in any case.

Which antbody subclass will be typically generated?

In principle, all subclasses of antibodies can be generated. The experimental setup must be adapted accordingly.

Are GERBU Adjuvants suitable for in vitro applications?

To our experience, GERBU Adjuvants are suitable for generating recombinant antibodies, chimeric antibodies, synthetic antibodies etc.

By using GERBU Adjuvants in vivo is it possible to generate monoclonal aswell as polyclonal antibodies against native epitopes of high quality and sufficient quantity in mice?

The type of bio-molecule that can bind to an active substance (target) and the tertiary/quaternary structure of the immunogen used have a strong influence on the immune response, i.e. the three dimensional shape of the antigens and their complex structures is very important.

I would like to use GERBU Adjuvants for commercial antibody production. Do I have to pay license fees in addition to the product price?

**commercial antibody production. Do I have** GERBU Adjuvants are developed for commercial use. There are no additional license **to pay license fees in addition to the** fees.

