

### Content and product information

**IFAVax** Incomplete Freund's adjuvant (IFA) is a water-in-oil emulsion that does not contain Mycobacteria.

### Description

**Incomplete Freund's Adjuvant (IFA)** and CFA are among the most widely used adjuvants in experimental immunology. IFA has been used for decades in practical veterinary vaccination.

IFAVax consists of a mixture of mineral oil and emulsifier in a ratio of 85% v/v oil (oleic acid – unsaturated C18 fatty acid) and 15% v/v emulsifier (mannide monooleate, an ester consisting of mannitol). Importantly, IFAVax is not a pre-formed emulsion and thus it must be mixed with an equal volume of aqueous solution of antigen and emulsified prior to use. Even if the mechanisms of action of oil emulsions are not totally understood, some evidences suggest a partial requirement for NOD2. Moreover, these emulsions are prone to cause cellular damage upon injection and thus, endogenous signals released during necrotic cell death may also contribute to their adjuvant activity. As opposed to CFA that induces principally a Th1 response, IFA that lacks mycobacterial components, primes a Th2 response and is thus less inflammatory. The engagement of Th2 outcome is due in part to the depot effect allowing a gradual and continuous release of antigen at the site of injection and an efficient rise in antibody titer. Freund's adjuvant is designed to provide continuous release of antigens necessary for stimulating strong persistent immune response. Freund's adjuvant is designed to provide continuous release of antigens necessary for stimulating strong persistent immune response.

IFA should be used responsibly and with care to avoid or minimize the adverse effects of excessive inflammation. For most applications, CFA is usually only necessary for the initial immunization, while IFA is the adjuvant of choice for subsequent immunizations.

### Use, handling and storage

*For Research Use Only. Not for use in humans. Not for use in diagnostic or therapeutic purposes.*

**Shipping conditions:** Room Temperature.

**Storage conditions:** 4°C.

**Shelf life:** 1 year from the date of purchase.

⚠ Do not freeze.

### Kit contents

**IFA0010:** 10 mL of IFAVax.

**IFA0050:** 5x10 mL of IFAVax.

**IFA0100:** 10x10 mL of IFAVax.

Certificate of analysis on demand.

### Method | Protocol

#### Recommendations before starting:

The inoculum should be free of extraneous microbial contamination; filtration of the antigen before mixing with the adjuvant is recommended.

Injections containing Freund's complete adjuvant should be given subcutaneously; it is recommended to divide the inoculum containing the adjuvant into fractions so that no more than **0.1 mL** is injected per site into mice or rats and **0.25 mL** per site for rabbits. If skin necrosis results while following these guidelines, future injections should be spaced farther apart.

1. For CFA only: vortex the vial to re-suspend the mycobacterium.
2. Prepare **1 mL** of IFAVax adjuvant in a plastic 3 cc luer-lock syringe.
3. Dilute the antigen mixture in saline buffer or phosphate buffer for a final immunogen concentration of **10-100 µg/100 µL** (sufficient for immunizing mice, rats and rabbits).
4. Prepare **1 mL** of antigen in a plastic 3 cc luer-lock syringe.
5. Form an emulsion:
  - a. Connect the two syringes using a double-ended locking connector.
  - b. Press syringe barrels back and forth, transferring contents from one syringe to the other. A white emulsion should form immediately.
  - c. Mix for **5 to 10 min**; a stable emulsion is produced.
  - d. The resulting emulsion should appear white, be stable and should not disperse when dropped into water.

NOTE: for smaller volumes, a prolonged mixing can be performed by vigorously pipetting or vortexing. For larger volumes use a tissue homogenizer to form the emulsion.

6. Separate the syringes and remove air before attaching the needle (**21 g** for mice or rats; **19 g** for rabbits).
7. Inject into the animal according to the table below; the volume depends on the site of injection.
8. It is necessary to separate multiple injection sites by a distance sufficient to avoid coalescence of inflammatory lesions.

NOTE: Preferential route of administration for Freund's adjuvant should be subcutaneous as it is the less disruptive. Other routes can be used: intramuscular, intradermal or intraperitoneal.

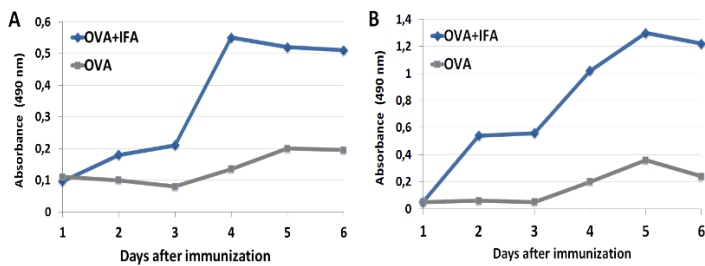
Typical routes of administration include subcutaneous (SC), intramuscular (IM), intradermal (ID), intraperitoneal (IP) or intravenous (IV).

Species	SC	IM	ID	IP	IV
Mice	0.1 mL/site 4 sites max.	NR	NR	0.25 mL max.	NA
Rats	0.1 mL/site 2 sites max.	NR	0.1 mL/site 8 sites max.	0.5 mL max.	NA
Rabbits	0.25 mL/site 8 sites max.	0.25 mL/site 2 sites max	0.1 mL/site 12 sites max.	NR	NA

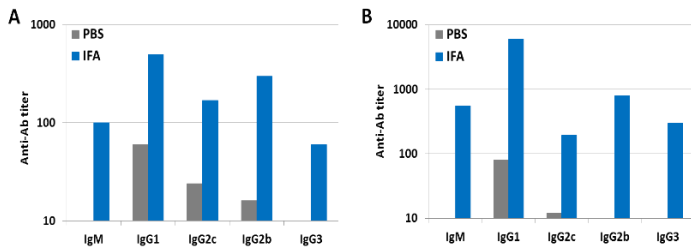
**Table 1:** Recommended volume of Freund's Adjuvant/Antigen emulsion per route of injection for different animal species (Adapted from Hendriken CFM et Hau J., 2003, in handbook of laboratory animal science vol I). NR= not recommended, NA=not acceptable.

## Results

Results presented below demonstrate the effect of IFA adjuvant on immune system response:



**Figure 1. Antibody response in mice immunized with ovalbumin (OVA) or OVA+IFA.** OVA-specific antibodies of all isotypes (A) or of IgG1 subclass (B) were revealed by ELISA several days after immunization with soluble OVA or OVA adsorbed with IFA (adapted from Albuquerque D et al. Vaccin 2011;20(2): 1-5).



**Figure 2. Comparison of serum antibody response to immunogen with or without IFA adjuvant.** Mice were immunized with 100µg antibody in PBS or in IFA. Titers of antibodies were determined on days 7 (A) and 14 (B) by ELISA (adapted from Gavin et al. Science 2006; 314).

## Example of protocol:

### Immunization of mice for production of antigen-specific antibodies

This rapid protocol describes immunization of mice with Freund's adjuvants/Ovalbumin antigen emulsion to induce high production of antibodies specific for an antigen. Mice are immunized with OVA emulsified in Complete Freund's Adjuvant (CFA), followed by a booster dose of protein emulsified in Incomplete Freund's Adjuvant (IFA).

1. Authorize the mice to acclimatize to the animal facility at least 7 days before immunization.
2. Dilute the antigen to a concentration of **100 µg** in **100 µL** of saline buffer.
3. Prepare OVA/CFA emulsion as described in protocol using 2 lock-luer syringes for a better emulsion stability.
4. Day 1: Inject mice with **0.1 mL** of antigen emulsified in CFA at two sites subcutaneously on the back of the mice (total 0.2 mL per mouse).

NOTE: Keep the needle inserted into the subcutaneous space for 10 to 15 seconds after each injection to avoid leakage of the emulsion. Withdraw the needle very precasiously.

5. Day 14: Administrate a booster injection of 0.1 mL of antigen emulsified in IFA subcutaneously at one site.

NOTE: it is recommended to collect a serum sample and test the antibody concentration 7 to 10 days after the boost injection. If the concentration is lower than expected, an additional booster dose of IFA emulsion may be given 14 days after.

6. Collect serum 7 to 14 days after administration of the last dose and purify antibodies.

## Related Products

Ref	Description
#AH0250	AlumVax Hydroxide 2%
#AP0250	AlumVax Phosphate 2%
#SQ0100	SqualVax, squalene oil-in-water emulsion
#CFA0100	Complete Freund's Adjuvant (CFA)
#CV02000	CaLiVax-DOTAP Adjuvant
#LV02000	LipoVax NTA(Ni)

## Purchaser Notification | Conditions of Sale

This product is sold in accordance with our general conditions of sale that you can find on our website: <https://ozbiosciences.com/content/3-terms-and-conditions>.