CFAVax

CFA - Complete Freund's Adjuvant

Product information and content

CFAVax Complete Freund's adjuvant (CFA) is a water-in-oil emulsion containing *Mycobacterium Tuberculosis* (1mg/mL).

CFAVax is available in three quantities: #CFA0010: 10 mL, #CFA0050: 5x10 mL & #CFA0100: 10x10 mL.

Storage and stability

Store CFA at +4°C away from light. Do not freeze.

Description

Complete Freund's Adjuvant (CFA) is a water-in-oil emulsion containing 1 mg per mL heat-killed dried *Mycobacterium tuberculosis*. CFA and IFA are among the most used adjuvants and have been used extensively in experimental immunology. Complete Freund's Adjuvants has been used for decades in practical veterinary vaccination.

CFAVax consists of a mixture of mineral oil and emulsifier in a ratio of 85% v/v oil and 15% v/v emulsifier. Each 10 mL CFA vial contains 10 mg *mycobacterium*. Importantly, the CFAVax is not a pre-formed emulsion and thus it must be mixed with an equal volume of aqueous solution of antigen and subsequently emulsified prior to use. The emulsifier is mannide monooleate, an ester consisting of mannitol as the hydrophilic residue and oleic acid (unsaturated C18 fatty acid) as the hydrophobic part. Even if the mechanisms of action of oil emulsions are still poorly 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. Immune response is dramatically enhance by the presence of the mycobacterial component that attracts macrophages and immune cells at the site of injection. CFA induces principally a Th1 response and can cause granulomas and an intense inflammatory reaction at the inoculation site.

Freund's adjuvant is designed to provide continuous release of antigens necessary for stimulating strong persistent immune response.

The use of CFA should be used responsibly and with care in order 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.

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 Complete Freund's Adjuvant should be given subcutaneously: it is recommended to divide the inoculum containing the adjuvant into fractions so that not more than 0.1 mL is injected per site in 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. Vortex the vial to re-suspend the mycobacterium.
2. Prepare 1 mL of CFAVax 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 vigourously 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 (21g for mice or rats; 19g 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.

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

SC = SC = Subcutaneous, IM = Intramuscular, ID = intradermal, IP = intraperitoneal, IV = IV = intravenous, N.R. = not recommended, N.A. = not acceptable

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).

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Results

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

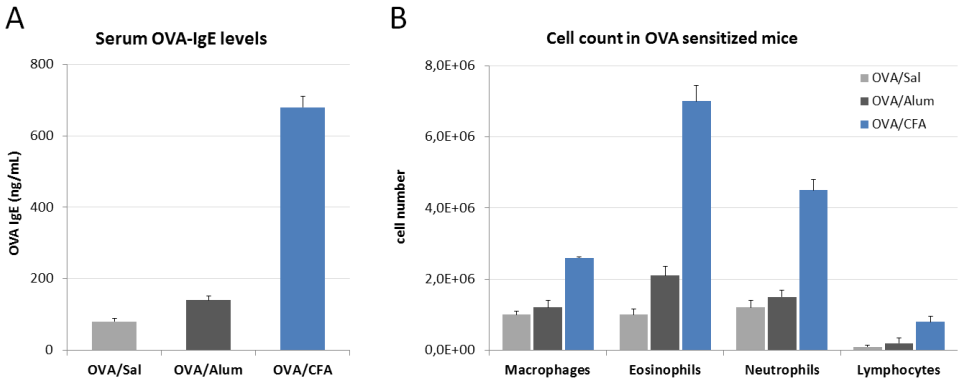

Figure 1. Immune response in mice immunized with ovalbumin (OVA) or OVA+CFA. (A) mean concentrations (mg/mL) in Mice serum of OVA-IgE after IP OVA immunization without adjuvant (OVA/Sal), alum (OVA/Alum) or CFA (OVA/CFA). (B) Mean total number of macrophages, eosinophils, neutrophils and lymphocytes in BAL fluid of mice after IP OVA immunization without adjuvant (OVA/Sal), alum (OVA/Alum) or CFA (OVA/CFA) (adapted from Nakada *et al*. Respiratory research 2014;15:90)

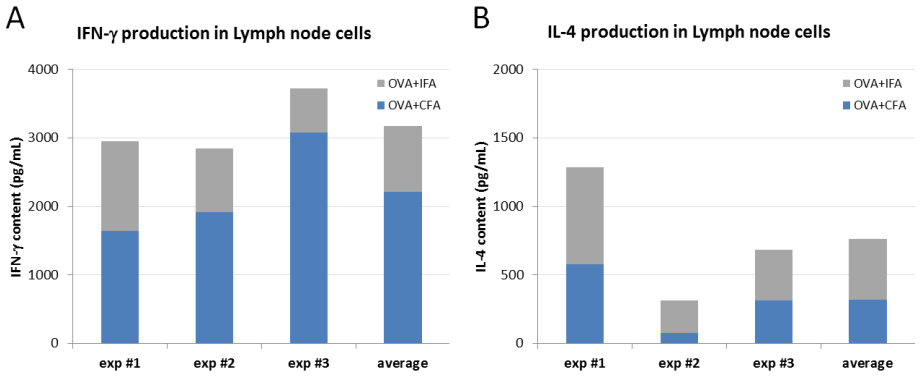

Figure 2. CFA adjuvant favors a strong Th1 response. BALB/c mice were immunized with OVA+IFA or OVA+CFA and draining lymph nodes were harvested 7 days later. (A) IFN-□ and (B) IL-4 production was measured by ELISA after 72H *in vitro* incubation with OVA. (adapted from Shibaki *et al*. Exp Derm. 2002;11:163-134).

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 precausiously.
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.