FT-314335

Freund's Adjuvants

The most popular adjuvant for animal immunizations

Product Description

Catalog #: 356155, 6x10ml
Name: **Freund's Complete Adjuvant (FCA)**
Water-in-oil emulsion and killed Mycobacterium (H37RA 1mg/ml)

Catalog #: 314335, 6x10ml
Name: **Freund's Incomplete Adjuvant (FIA)**
Water-in-oil emulsion

Storage: Store in a cooler at 2-8 °C. Do not freeze. (z)

Introduction

Adjuvants are non-specific stimulators of the immune response. When mixed with an antigen, they help to deposit or sequester the injected material. In addition, they cause a dramatic increase in the resultant antibody response. Freund's adjuvant was popularized and remain the most largely for immunization at first intention, despite several drawbacks.

Directions for Use

Protocol 1:

FCA and FIA should be kept at room temperature before use. Wear gloves.
FCA is used for initial injections of antigen into animals to enhance the immune response. Vortex or shake before use. FIA is used for further boosts.

1/ **Mix equal volumes of immunogen** (preferably in saline) and Freund's adjuvant.
-Final immunogen concentrations of 20-50 µg/100 µl are generally be sufficient to immunize mice, and rats, up 100µg in rabbits and up 0.5-1mg in goats. However, considerable variations of immunogenicity may be observed, and quite different quantity could be found in the literature.

-The quality of mixing is important for favoring reproducible immunization in an animal, between animals, and for each immunization. **Vigorous and prolonged mixing is needed**. The principal difficulties result from difference in viscosity/density between the sample and the adjuvant.

For small volumes the emulsion can be made in a tube: pipette the adjuvant in the tube first, then, while vortexing, add an equal volume of the antigen solution. Vortex vigorously until a thick emulsion forms. A 10-15min mixture is required for obtaining optimally a thick emulsion. A drop of the emulsion should not disperse if thrown in saline water.

For intermediate volumes, two syringes connected through a luer fitting (ideally via 3-way valve to remove all air) provide an efficient operating. Take the desired amount of antigen solution into a glass syringe (less than half the syringe volume). Take an equal volume of the adjuvant into another glass syringe and adapt to the other syringe (purge air via the 3-way valve). Carefully depress the plunger from the antigen solution first, pushing the antigen into the oil of the adjuvant. Reverse operating, until the plungers are difficult to push.

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For large volumes, emulsion can be performed using a tissue homogenizer. Add the adjuvant to the homogenizer first. Run the homogenizer for a short time with the adjuvant first to coat the inside, then add an equal volume of the antigen solution and run until a thick emulsion forms.

2/ Inject the emulsion into animal by SubCutaneous route or IntraDermal route.
Note: Use an appropriately sized needle.
Attention should be paid to remove the air from the syringe before injection, and operate rapidly.
ID route does not suit to rodents (mice).
Other routes (IntraPeritoneal, or IntraGanglionar) are not recommended.

Technical and Scientific Information

- Adjuvant **composition**

AFC is a Water-in-oil emulsion and killed Mycobacteria, while AFI does not contain Mycobacteria.
They are manufactured under controlled procedures for reproducible quality, of immunogenic grade.
It is prepared from non-metabolizable oils (paraffin oil and mannide monooleate).

- Adjuvant **effects**

First developed by Jules Freund in the 1940's, Freund's Adjuvant is designed to provide continuous release of antigens necessary for stimulating a strong, persistent immune response.
Freund adjuvants are mixed to antigens before injection to animals for production of specific antibodies. They stimulate the immunization through numerous factors. One important effect is to elicit an inflammation site and it's ability to improve the presentation of antigen to immuno-competent cells. As a result, the immune response if more rapid, strong (higher titer of antibodies), and longer lasting. They were popularized worldly and have become the more commonly used adjuvants in any animal warehouses. However, some good practices should be observed for their use (http://www.ccac.ca/french/gui_pol/policies/IMMUNO.HTM) and it should not be used in Horses. FCA elicits effectively several drawbacks. Additional information could found at
http://www.unimelb.edu.au/research/ethics/animal/docs/Policy_No_2_Polyclonals_Feb_2002.rtf
For production of antibodies to study protein conformation, antigen preparation should not denature protein antigens. This was shown for emulsification of hemoglobin in AFC, by electron paramagnetic resonance to observe directly the protein in situ in the opaque emulsions (J Immunol. 1976 Feb;116(2):270-2).

- AFC **drawbacks**

-As main disadvantage, Freund's Adjuvant elicits severe inflammations to animals. FCA can cause granulomas, inflammation at the inoculation site and lesions. The mycobacteria in Complete Freund's attracts macrophages and other cells to the injection site which enhances the immune response. For this reason, the Complete Freund's Adjuvant is used for the initial injections. To minimize side effects, Incomplete Freund's Adjuvant is used for the boosts.
-Some investigators are highly sensitive to FCA and are at considerable risk of developing an acute hypersensitivity reaction as well as a severe local reaction at the site of injection should accidental inoculation occur. For this reason caution must be exercised in operating.
-Finally, emulsion is not always easy to prepare and no well stable, and it is not very easy to infect with syringe/needle (gripping), introducing some additional variability to immunization response.

To that purpose, alternative adjuvants were proposed, such as our Titer Max adjuvants.

- **'Adjuvants'** and **'Carriers'**

There is sometime confusion between 'adjuvants' and 'carriers'. Small molecules are often not immunogenic by themselves, and even (referred as haptens) do not become immunogenic when injected with an adjuvant. However haptens can become able to elicit an immune response after conjugation to a big molecule, carriers such as BSA or our improved MaxiBind carrier proteins. For such hapten-carrier immunogenic conjugates, the immune response will also be enhanced by adjuvant, as AFC/AFI.
That is to say, adjuvant will not render haptens immunogenic, but will increase their immune response.

Literature

Harlow, E. and Lane, D. (1988). Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 56-100.
Male, D., Champion, B. and Cooke, A. (1987). Advanced Immunology. J.B. Lippincott Co., Gower Medical Publishing, London 8.1-8.8.
Sell, S. (1987). Immunology immunopathology and immunity. Elsevier, New York 69-78.

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Legals

For in vitro R&D use only

Other Information

Related / associated products

Titer Max adjuvants (compared with AFC: no induction of hypersensitivity to animals; easier to inject with a syringe; safer)
MaxiBind carrier proteins (superior coupling ratio)

Please contact Uptima – Interchim for any other information

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