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AdjuLite Freund's Adjuvants

Catalog Number	Description	Quantity	Price
A5001	AdjuLite® Freund's <i>Complete</i> Adjuvant, Includes 5 syringe connectors.	6 x 10 ml	\$126.00
A5002	AdjuLite® Freund's <i>Incomplete</i> Adjuvant, Includes 5 syringe connectors.	6 x 10 ml	\$114.00
A5003	AdjuLite® Freund's Adjuvant Combo-Pak, Includes 5 syringe connectors. AdjuLite Freund's <i>Complete</i> Adjuvant, AdjuLite Freund's <i>Incomplete</i> Adjuvant,	1 x 10 ml 5 x 10 ml	\$120.00

The AdjuLite Advantage

Fluid emulsion, injects and spreads easily
Superior presentation of the immunogen
Conserves valuable antigens, lower doses required
Single site injection, less pain and stress for the animals
Unsurpassed stability of the emulsion enhances the immune response
High purity components virtually eliminate injection site abscesses and irritation

Appearance: AdjuLite *Incomplete* Freund's Adjuvant, amber liquid
AdjuLite *Complete* Freund's Adjuvant, amber liquid containing dried cells

Store refrigerated (2-8°C), do not freeze. Product is shipped at ambient temperature. These adjuvants are for use in laboratory animals only, not for use in humans. Do not inject intravenously.

Visit our web site for additional antibody production information.

Introduction

Adjuvants are materials used to stimulate and enhance the immune response to an immunogen. By mixing the immunogen with the adjuvant to form a stable emulsion, a sustained presentation of the antigen is achieved. This depot effect results in improved immune response and higher serum antibody levels.

Freund's Adjuvants are used to form stable water-in-oil emulsions which continuously present the antigen to the immune system, resulting in high and long-lasting antibody response.

Freund's adjuvants consist of a mixture of mineral oil and an emulsifier mannide monooleate. AdjuLite *Complete* Freund's Adjuvant contains 1 mg/ml heat-killed and dried *Mycobacterium tuberculosis* H37Ra. The mycobacteria stimulate the immune system and enhance the immune response.

Your animals and your research deserve AdjuLite Adjuvants.

The AdjuLite Advantage

AdjuLite *Complete* Freund's Adjuvant is used for the first immunization, and AdjuLite *Incomplete* Freund's Adjuvant is used for all subsequent immunizations. AdjuLite Freund's Adjuvants are prepared from highly refined, ultra-light mineral oils which form a stable but fluid emulsion. This fluid emulsion is injected subcutaneously and spreads out under the skin, providing superior presentation of the immunogen to the immune system.

Antigens in saline or phosphate buffers are typically mixed with an equal volume of the adjuvant to form an emulsion.

Primary immunization.

ANTIGEN: Prepare 1 ml of antigen in a plastic 3 cc luer-lock syringe. Antigens diluted in saline or phosphate buffers are preferred. The amount of protein or conjugated peptide used for the primary immunization of rabbits is typically 200 µg. This amount can be adjusted depending upon availability and immunogenicity of the antigen. Doses as small as 5 µg have been used to elicit an immune response. Mice and rats can be immunized with 20-50 µg doses. Protein bands cut from gels are also effective immunogens. For a rabbit, add 200-400 µl of gel to the syringe and then add saline to a volume of 1 ml. Mice and rats would receive less gel. The amount of immunogen in the gel should be similar to the dose of soluble antigens.

ADJUVANT: Prepare 1 ml of adjuvant in a plastic 3 cc luer-lock syringe. For the first injection, use AdjuLite *Complete* Freund's Adjuvant. Swirl the vial to suspend the mycobacteria prior to withdrawing the adjuvant.

EMULSION: Connect the two syringes with the female luer thread coupler supplied with the adjuvant kit. These connectors can be washed and used repeatedly. Push the antigen and adjuvant back-and-forth between the syringes. A white emulsion will form immediately, but continue to mix for several minutes to form an emulsion with maximum stability. Separate the syringes, remove any air in the syringe, and attach a 19 ga x 1 inch needle for rabbits, or a 21 ga x 1 inch needle for mice or rats. The stability of the emulsion can be evaluated by leaving the syringe in the refrigerator overnight and looking for separation of a clear phase from the white emulsion. If properly mixed, no separation of the emulsion will be seen for several weeks. *A stable emulsion is essential for the production of high titer antibodies. If the emulsion separates soon after immunization, then presentation of the antigen is short-lived, and antibody response will be poor.*

INJECTION: Inject the 2 ml of emulsion subcutaneously, beneath the loose skin at the back of the neck of the rabbit. Pinch and pull up the skin and inject through the skin into the pocket between the skin and muscle. The fluid nature of the AdjuLite Adjuvants allows a single injection to be given. The emulsion will spread out under the skin and give extended antigen presentation. Mice and rats will typically receive about 0.3 to 0.5 ml of emulsion, administered in a similar manner. *Avoid intradermal administration of the emulsion. This will cause unnecessary pain and irritation to the animal, and is not needed due to the superior presentation properties of the AdjuLite Adjuvant System.*

Subsequent immunizations.

The primary difference between the first injection and subsequent injections is the choice of adjuvant and the dose of immunogen. AdjuLite *Complete* Freund's Adjuvant is used only for the first immunization. AdjuLite *Incomplete* Freund's Adjuvant is used for all subsequent immunizations. The dose used for secondary immunizations is usually half of the primary dose. Follow the procedures described above to prepare the immunizations.

REFERENCES

- Harlow, E. and Lane, D., *Antibodies a Laboratory Manual*, (Cold Springs Harbor Laboratory, 1988).
- Freund, J., 1956, The mode of action of immunologic adjuvants. *Adv. Tuberc. Res.*, 7:130-148.
- Herbert, W. J., 1967, Methods for the preparation of water-in-oil, and multiple, emulsions for use as antigen adjuvants; and notes on their use in immunization procedures. In: *Handbook of Experimental Immunology*, D. M. Weir and Blackwell, Eds., 1207-1217.
- Bomford, R., 1980, The comparative selectivity of adjuvants for humoral and cell-mediated immunity. II. Effect on delayed-type hypersensitivity in the mouse and guinea pig, and cell-mediated immunity to tumor antigens in the mouse of Freund's incomplete and complete adjuvants, Alhydrogel, *Corynebacterium parvum*, *Bordetella pertussis*, muramyl dipeptide and saponin. *Clin. Exp. Immunol.*, 39:435-441.