



Product Information

FREUND'S ADJUVANT, COMPLETE AND INCOMPLETE

Product Number **F 5881 AND F 5506**

Storage Temperature 2-8 °C

Product Description

Appearance

F 5881 Clear amber liquid containing particulate matter (dried cells)

F 5506 Clear amber liquid

Freund's Adjuvant is one of the most commonly used adjuvants in research. It is used as a water-in-oil emulsion. It is prepared from non-metabolizable oils (paraffin oil and mannide monooleate). If it also contains killed *Mycobacterium tuberculosis* it is known as Complete Freund's Adjuvant. Without the bacteria it is Incomplete Freund's Adjuvant. 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^{1,2,3}. The main disadvantage of Freund's Adjuvant is that it 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.

For comparisons of different adjuvant systems, see references 4 and 5.

Reagents

Each ml of F 5881 contains 1 mg of heat-killed and dried *Mycobacterium tuberculosis* (strain H37Ra, ATCC 25177), 0.85 ml paraffin oil and 0.15 ml of mannide monooleate.

Each ml of F 5506 contains 0.85 ml of paraffin oil and 0.15 ml of mannide monooleate.

Precautions and Disclaimer

Please consult the Material Safety Data Sheet for handling recommendations before working with this material.

Storage/Stability

Store in a cooler at 2-8 °C. Do not Freeze.

Procedure

1. If using Complete Freund's Adjuvant, vortex or shake to resuspend the *Mycobacterium*.
2. Mix antigens (preferably in saline) with an equal volume of the adjuvant to form an emulsion. In order to do this, vigorous and prolonged mixing is needed. There are at least three methods which can be used to accomplish this:

For small volumes the emulsion can be made in a tube. Pipet the adjuvant in the tube first. Then, while vortexing, add an equal volume of the antigen solution. Vortex vigorously until a thick emulsion forms.

For intermediate volumes, use two syringes connected through a luer fitting. Ideally, a 3-way valve should be used. Take the desired amount of antigen solution into a glass syringe. The volume should not fill more than half the syringe. Take an equal volume of the adjuvant into another glass syringe. Remove all air and connect the syringes through the luer fitting to the 3-way valve. Adjust the 3-way valve such that the connection is open between the two syringes. Carefully depress the plunger from the antigen solution first, pushing the antigen into the oil of the adjuvant. Alternately push the plungers, mixing the adjuvant and the antigen solution into an emulsion. Continue until the plungers are difficult to push.

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

3. The resulting emulsion should be very thick and a drop of it should not disperse if tested by placing on the surface of a saline solution.
4. Transfer the emulsion to a syringe (or remove one syringe from the luer fitting if using the two-syringe method). Remove all the air. Add an appropriately sized needle. The samples are now ready for injection.

References

1. Freund, J. and McDermott, K., Proc. Soc. Exp. Biol. Med., **49**, 548-553 (1942)
2. Freund, J., Ann. Rev. Microbiol., **1**, 291 (1947)
3. Freund, J., Adv. Tuberc. Res., **7**, 130 (1956)
4. Bennett, B. et al., J. Immuno. Meth., **153**, 31-40 (1992)
5. Deeb, B.J. et al., J. Immuno. Meth., **152**, 105-113 (1992)
6. Harlow, E. and Lane, D., Antibodies A Laboratory Manual, (Cold Spring Harbor Laboratory, 1988)

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