

Product Information

TiterMax® Classic Adjuvant

Catalog Number **H4397**

Storage Temperature 2-8 °C

Product Description

The form and stability of an immunization emulsion contribute strongly to the effectiveness of an oil emulsion vaccine. Emulsions are mixtures of two immiscible fluids, one of which is suspended as small drops inside the other, and are stabilized by surface-active agents. There are two principal kinds of emulsions, water-in-oil and oil-in-water. In the former, oil forms the continuous phase that surrounds small droplets of water, the discontinuous phase. Water forms the continuous phase in an oil-in-water emulsion. The water-in-oil formulations, such as TiterMax Classic Adjuvant and Freund's, are the most powerful adjuvants in most protocols.

TiterMax Classic Adjuvant contains three essential ingredients: a block copolymer, CRL89-41, squalene (a metabolizable oil) and a unique microparticulate stabilizer.

It has been especially formulated with squalene to produce stable water-in-oil emulsions with up to 90% water. It is considerably easier to emulsify than Freund's Adjuvant. The resulting emulsion is less viscous, making it easy to inject through small needles.

Recommended Materials

- Two 2.5 ml all-plastic, Catalog No. Z248010, or siliconized glass, luer lock syringes.
Note: Syringes should be siliconized glass or all-plastic. Plastic syringes with rubber pistons contain a lubricant that fails in the presence of TiterMax Classic Adjuvant and causes syringes to stick.
- One 18 gauge needle.
- One Stopcock, 3-way Luer Lock, plastic, Catalog No. S7521, or stainless steel stopcock,

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Interfering Reagents

Reagents high in surfactants may interfere with the emulsifying capacity of TiterMax Classic. It has been found that urea in concentrations > 1.0 M and other similar materials significantly reduce the ability to form an emulsion.

Storage/Stability

Store TiterMax Classic Adjuvant at 2-8 °C. The water-in-oil emulsion can be stored as the un-emulsified antigen would be stored, i.e. room temperature, 2-8 °C, -20 °C or -70 °C, for as long as the antigen is stable. Upon storage, approximately 20% of the oil will disassociate from the emulsion. Re-emulsify when ready to use again.

Recommended Injection Protocol

For mice: 4 divided doses of 10 µL each into 4 subcutaneous or preferably intramuscular sites.

Recommended Antigen Concentration

10-100 µg/dose, depending on the antigen.

Procedure: Two-Syringe, 3-Way Stopcock

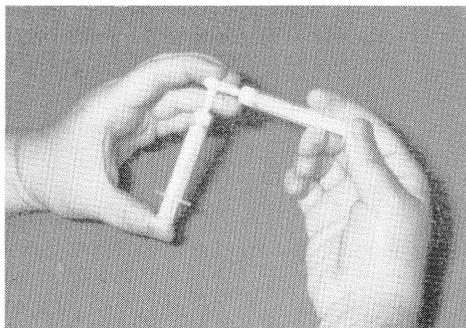
Note: Prior to preparation, warm TiterMax Classic Adjuvant to room temperature and vortex for 30 seconds. Make sure that TiterMax Classic Adjuvant is a homogenous suspension before proceeding with emulsification.

To prepare 1.0 mL of the recommended water-in-oil TiterMax Classic Adjuvant emulsion, 0.5 mL of aqueous antigen is required. Good immune responses have been achieved with water to TiterMax Classic Adjuvant ratios of 10:90 but the 50:50 water-in-oil emulsion is usually optimum. Each vial of TiterMax Classic Adjuvant contains 750 µL which will provide enough product to load a syringe with 500 µL.

1. After the TiterMax Classic Adjuvant has been vortexed, load one syringe with 0.5 mL TiterMax Classic and load the second syringe with 0.25 ml antigen in aqueous medium. Set aside the other 0.25 ml of antigen.

Note: It is important to add the aqueous phase to the TiterMax Classic in at least 2 small volumes.

2. Connect the two syringes via the 3-way stopcock. Mix the TiterMax Classic Adjuvant with the antigen by forcing the materials back and forth through the stopcock for approx. 2 minutes
See Figure 1.



1. Figure 1

Note: It is important to push the antigen into the TiterMax Classic Adjuvant syringe first, so that the aqueous phase enters the oil phase rather than vice versa.

3. After approximately 2 minutes, a meringue-like water-in-oil emulsion forms. Push all of the emulsion into one syringe and disconnect the empty syringe.
4. Load the empty syringe with the remaining 0.25 mL aqueous antigen solution. Reconnect the syringes and emulsify for another 60 seconds.

Note: Again, first push the antigen into the water-in-oil emulsion. Care must be taken in holding the syringes together since the oil may lubricate and loosen the connection. It is preferable to use a luer lock syringe.

5. Push all of the emulsion into one syringe and disconnect the empty syringe.
6. To quickly and easily test whether your TiterMax Classic Adjuvant - antigen emulsion is ready to use, expel a tiny drop onto the surface of water. It should expel from the syringe with a consistency similar to whipped cream and should hold together on the surface of water. In the event that the emulsion disperses on the surface of the water, reconnect the syringes and emulsify for another 2 minutes.

Note: Other emulsification protocols are available upon request.

Boosting

Since TiterMax Classic Adjuvant produces high antibody titers without boosting, a single injection may produce sufficient antibody. It is important to be aware that injecting antigen into an animal with high antibody titers can elicit a local Arthus reaction. If primary titers are high, boost with a very low dose of TiterMax Classic Adjuvant or antigen in saline to avoid local Arthus reactions. Experience has shown that injection of multiple small doses is superior to a single large dose injection.

References

1. Hunter, R., et al., *J. Immunol.*, **127**, 1244 (1981).
2. Hunter, R.L., and B. Bennett, *Scand. J. Immunol.*, **23**, 287 (1986).
3. Hunter, R.L., and B. Bennett, in *Immunopharmacology of Infectious Diseases: Vaccine Adjuvants and Modulators of Non-Specific Resistance*, J.A. Majde, ed., Alan R. Liss, Inc., New York, p. 181 (1987).
4. Hunter, R.L., et al., in *Immunological Adjuvants and Vaccines*, G. Gregoriadis, A.C. Allison, G. Poste, eds., Plenum Press, New York, p. 133 (1989).
5. Hunter, R.L., and B. Bennett, *J. Immunol.*, **133**, 3167 (1984).

TiterMax is a registered trademark of TiterMax USA, Inc..

PHC 10/10-1

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.