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Product Information

TiterMax® Gold Adjuvant

Catalog Number **T2684**
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, which 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® Gold and Freund's, are the most powerful adjuvants in most protocols.

TiterMax Gold Adjuvant contains three essential ingredients: a block copolymer, CRL-8300, squalene (a metabolizable oil), and a unique microparticulate stabilizer. It has been especially formulated with squalene to produce stable water-in-oil emulsions. It is considerably easier to emulsify than Freund's Adjuvant. The resulting emulsion is less viscous, making it easy to inject through small needles.

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

Equipment Required but Not Provided
- Two luer lock syringes:
  Two all-plastic, 5 mL syringes
  Catalog Number Z248010
  or
  Two all-plastic, 3 mL syringes
  Catalog Number Z248002
  Note: Syringes should be siliconized glass or all-plastic. Plastic syringes with rubber pistons contain a lubricant that fails in the presence of TiterMax Gold Adjuvant and causes syringes to stick.
- One 18 gauge needle
- One Stopcock, 3-way Luer Lock, plastic, Catalog Number 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.

Storage/Stability
Store the product at 2–8 °C.

The water-in-oil emulsion with antigen can be stored as the unemulsified 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, ~20% of the oil will disassociate from the emulsion. Re-emulsify when ready to use again.

Procedure
Two-Syringe, 3-Way Stopcock
The recommended injection protocol for mice is 4 divided doses of 25 µL each into 4 subcutaneous or preferably intramuscular sites.

The recommended antigen concentration is 10–100 µg/dose, depending on the antigen.

To prepare 1.0 mL of the recommended water-in-oil emulsion, 0.5 mL of aqueous antigen is required. A 50:50 water-in-oil emulsion is usually optimal. Each vial of TiterMax Gold contains enough product to load a syringe with 500 µL two times.

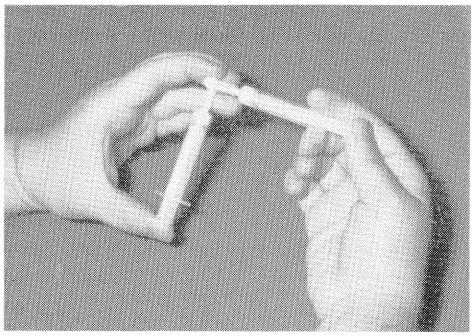
Note: Prior to preparation, warm TiterMax Gold Adjuvant to room temperature and vortex for 30 seconds. Make sure it is a homogenous suspension before proceeding with emulsification.

1. After the TiterMax Gold Adjuvant has been vortexed, load one syringe with 0.5 mL of TiterMax Gold Adjuvant and load the second syringe with 0.25 mL of 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 Gold Adjuvant in at least 2 small volumes.

2. Connect the two syringes via the 3-way stopcock. Mix the TiterMax Gold Adjuvant with the antigen by forcing the materials back and forth through the stopcock for ~2 minutes, see Figure 1.
   Note: It is important to push the antigen into the TiterMax Gold Adjuvant syringe first, so that the aqueous phase enters the oil phase rather than vice versa.

   Figure 1.
   Emulsion Formation



3. After ~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 of 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 Gold-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 Gold 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 Arthrus reaction. If primary titers are high, boost with a very low dose of TiterMax Gold Adjuvant or antigen in saline to avoid local Arthrus reactions. Experience has shown that injection of multiple small doses is superior to a single large dose injection.

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