

TiterMax™ adjuvants

Introduction

A non toxic, stable and reliable adjuvant, alternative and even superior to classic adjuvants (Freund, Alum...)

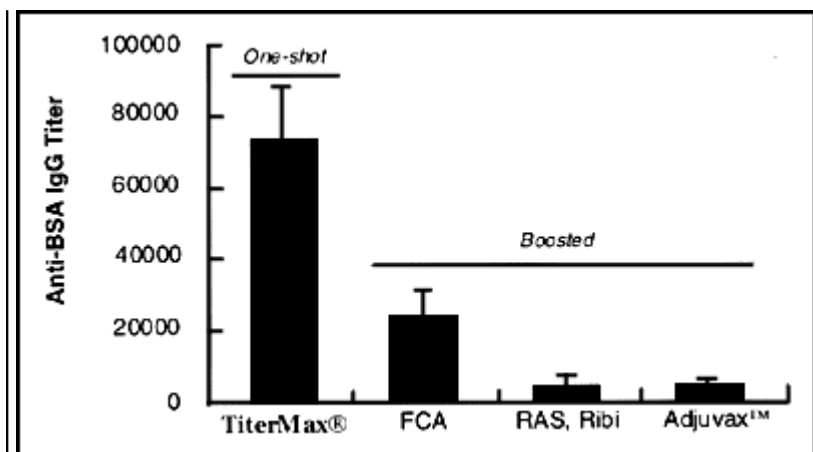
cat.number : Name:	898250 R-1 , 0.5ml TiterMax Classic r	995850 G-1 , 1ml TiterMax Gold	<i>For comparison</i> Complete Freund's Adjuvant
Vehicule	Squalene		Mineral oil
Adjuvant	Copolymer CRL-8941	Copolymer CRL-8300	Tuberculosis mycobacterium
Emulsifier	Non-ionic tensio-active		Non-ionic tensio-active
Stabilizer	Microparticules covered with copolymer		none
Emulsion formed	water in oil		water in oil
Security	Transitory inflammation		Severe transitory inflammation
Hypersensitivity	No induction, or limited		+ OR - (granulomatous resp., DTHS)
Efficiency	exceeds often the Freund adjuvant with boost	even better than classic TiterMax for some antigens	Powerful adjuvant
Manufacturer	Strictly controlled manufacturing process		Variable quality according to manufacturers, bacteria lots...
Preparation / use	Similar to Freund, created a more stable emulsion		Little stable emulsion
Storage	Easier to inject with a needle at +4°C (L)	at +4°C (L)	Difficult to inject at +4°C (L)

Freund's Complete Adjuvant (FCA) has been used for over 50 years for producing antisera in animals. The severity of its toxicity was recognized immediately, but attempts to find a less toxic and equally effective alternative have not been successful. With advances in many areas of the biological sciences and increasing concern for the welfare of experimental animals, there is increased pressure to ban or restrict the use of FCA.

TiterMax® is an adjuvant used to produce cell mediated and humoral responses in research animals. TiterMax® was developed to meet the specific needs of investigators for an immunoadjuvant that is at least as effective as Freund's Complete Adjuvant, safer and easier to use. It is an attractive alternative to Freund's Complete Adjuvant for use by researchers in inducing antibody to diverse antigens. The key to the potency of TiterMax® lies in the immunostimulatory activity of its components combined to the fact that it forms a stable water-in-oil emulsion. TiterMax® contains three essential ingredients: A proprietary block copolymer CRL-8941, squalene, a metabolizable oil, and a unique microparticulate stabilizer. Like Freund's Complete Adjuvant, TiterMax® can be used with a wide variety of antigens because it can entrap any antigen in a water-in-oil emulsion. TiterMax® aids in the antigens effective presentation to the immune system without the toxic effects of Freund's Complete Adjuvant.

Figure 1. Effectiveness of various adjuvants in rabbits.

Rabbits were immunized with 50 µg of bovine serum albumin (peptide conjugated) in various adjuvants according to the manufacturers' instructions. The TiterMax® group was not boosted, while the others were boosted according to the recommended procedures. Serum IgG antibody titers (mean±sem) were measured by ELISA after 8 weeks.



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TiterMax Gold can be used with a wide variety of antigens that can be entrapped in a water-in-oil emulsion. Like TiterMax Classic, TiterMax Gold does not contain mineral oil, proteins, polysaccharides or other microbial products, thereby minimizing or eliminating the undesirable side effects caused by Freund's Complete Adjuvant.

A single injection of TiterMax® can produce significantly higher titers than two or more injections of antigen in other commercially available adjuvants. Based on our experience, TiterMax® can be expected to perform at least as well as FCA with almost any antigen. With many antigens, TiterMax® performs significantly better. Thus, it is an attractive alternative to FCA.

Technical Information

General benefits of TiterMax adjuvants

- **Toxicity / Hypersensitivity**

TiterMax adjuvant is free of proteins, peptides or bacterial/vegetal compounds. It is completely non-toxic to user and animals. It induce a moderate transitory inflammation in animals, when Freund's adjuvant, especially CFA, induce **▪ a severe inflammation ▪ No induction of Hypersensitivity Granulomatous Response ▪ Minimum Induction of Delayed Type HyperSensitivity**. Thus TiterMax are an excellent alternative for ethical reasons, when animal uses in R&D and for biotechnologies are more and more regulated.

While non-toxic, TiterMax classic may make the animals susceptible to Arthus reaction at the site of immunization with very high ab titers, particularly when boosting. This can be minimized by :

- injecting Ag emulsion in multiple site using small volumes
- with soluble Ag for boosting (no adjuvant)

- **Immunogenic effect**

TiterMax without boost often exceeds the Freund adjuvant with boost. It overcomes the efficiency of other synthetic adjuvants, as Alum, Ribi... High titers are observed within 12 weeks without boost, thanks to proprietary immunomodulators.

Like FCA, TiterMax® can entrap any antigen in a water-in-oil emulsion; which is critical for dependable adjuvant activity. In contrast, many new adjuvants simply bind antigens by adsorption. If the antigens do not adsorb, they seldom induce high antibody titers. TiterMax® combines the benefits of a water-in-oil emulsion with those of potent block copolymer adjuvants, yielding highly reproducible results.

- **Use**

TiterMax is more easy to use than Freund adjuvant, because it is easier to pipette (homogeneous solution), the emulsion is more stable allowing increased time of handling and more consistent efficiency from animal to animal, do no grip the plastic piston of syringes, and is easier to inject.

- **Reliability, consistency**

Because TiterMax is synthetic, lots are very reproducible when Freud adjuvant manufacturer is subjected to variations of quality depending on mycobacterium lots, manufacturer process. Furthermore, the more stable formed emulsion permit better reproducibility from injection to injection, between each animals or between each boost.

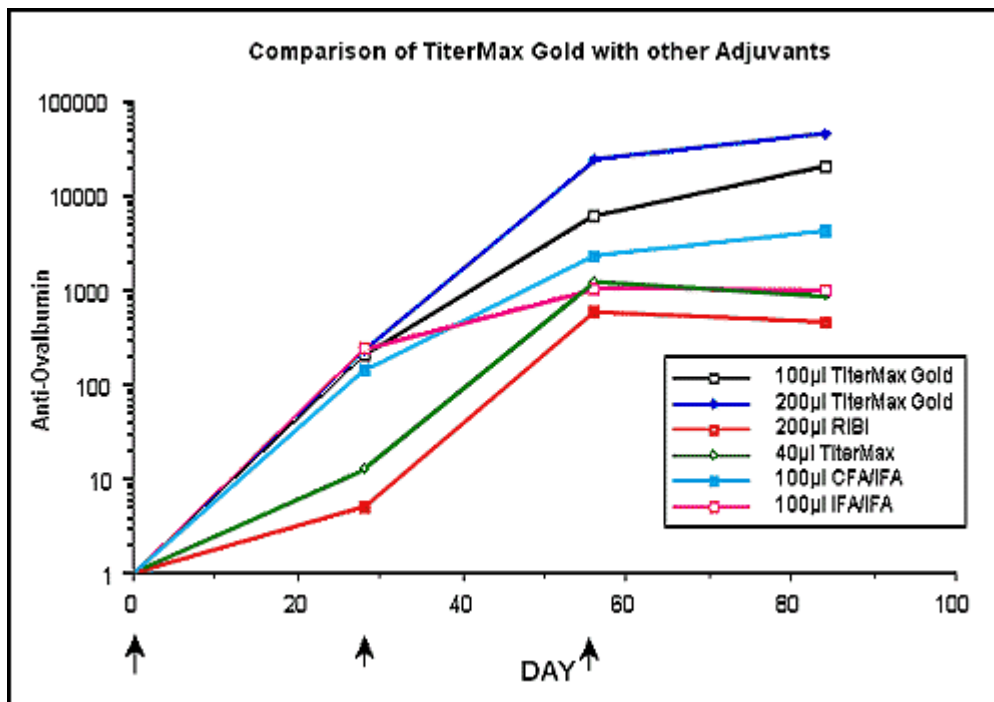
- **Immunisation potency**

Comparison data (see fig.1 above)

Studies were conducted using ovalbumin with various research adjuvants. Six to eight-week old female C57BL/6 mice (10 per group) were immunized with a constant dose of 25µg of ovalbumin with each commercial adjuvant. Optimal dosing regimens for TiterMax Gold were determined. Secondary immunizations were with antigen and adjuvant. All commercial adjuvants were prepared, injected, and boosted according to the manufacturers protocols. Serum samples were obtained at various times after immunization (days 28. 56. 84) and IgG Ab titers against ovalbumin were determined using a microtiter plate ELISA. Data is expressed as units/ml against an in-house standard.

TiterMax Gold (G-1) versus other adjuvants

TiterMax Gold injected intramuscularly, outperformed the other commercial adjuvants at all time points (days 28, 56 and 84). TiterMax Gold was found to be effective following single and multiple immunizations. For polyclonal antibody production, we recommend immunizing mice with a 100 or 200 µl dose (TiterMax Gold and antigen), injected intramuscularly into each hind quadricep.



TiterMax classic (R-1) versus TiterMax Gold (G-1)

Considering composition, TiterMax Classic and TiterMax Gold differ mainly by the block copolymer (see table 1). TiterMax Classic contains also a small amount of silica material, while the Gold does not.

Considering efficiency, both TiterMax adjuvants have in general excellent efficiency in comparison to Freund's adjuvant and others. Each antigen has to be evaluated with any adjuvant, because there are many parameters than can affect differently efficiency of adjuvants, including, route of injection, frequency, antigen nature, quantity, ratio of adjuvant/antigen... So, we do not have general recommendations, just suggestions and examples.

* TiterMax may be chosen in first intention, because it shows excellent efficiency in most applications and is more economic than TiterMax Gold.

* TiterMax Gold is a good choice if Ab responses are lower than expected with TiterMax Classic. TiterMax Gold has shown effectively better responses than TiterMax Classic and CFA in several applications.

Copolymer Adjuvant

CRL-8941 is a synthetic polymer composed of blocks or chains of hydrophobic polyoxypropylene (POP) and hydrophilic polyoxyethylene (POE). The adjuvant activity of block copolymers was described in 1981 by Hunter et al. CRL-8941 was selected for its adjuvant activity, which is typical of other block copolymers and its ability to form stable water-in-oil emulsions with squalene.

Directions for Use

1- Preparation of the emulsion

A 50:50 water-in-oil emulsion can usually be stored at room temperature, 4° C, -20° C, or -70° C for as long as your antigen is stable. Upon storage, approximately 20% of the oil will disassociate from the emulsion. You may leave the emulsion in a syringe and simply re-emulsify when you are ready to use again for injecting. The stability of an emulsion will depend upon the inherent stability of the antigen.

2- Examples of successful dosing regimens

Suggested dosage with TiterMax™ classic (#R-1)

Species	Method of injection	Number of injection	Volume of injection	Injection place
Mouse	IM	1	100µl	Each quadriceps
	SC	1	100µl	Base of the tail
Rat	IM	2	100µl	Each quadriceps
Guinea-pig	IM	2	100µl	Each quadriceps
	SC	4	100µl	On each shoulder and each quadricep
Rabbit	IM	2	100µl	Each quadriceps
	SC	4	200µl	On each shoulder and each quadriceps
Chicken/Turkey	IM	2	100µl	In each pectoral muscle In the neck
	SC	1	200µl	
Cat/Dog	IM	2	200µl	Each quadriceps Along the neck
	SC	1	200µl	
Monkey Rhesus	IM	2	200µl	each quadriceps
	SC	4	100µl	Inner thigh

Suggested dosage with TiterMax™ Gold (#G-1)

Species	Method of injection	Number of injection	Volume of injection	Injection place
Mouse	IM	2	20µl	Each quadriceps
	SC	1	40µl	Base of the tail
Rat	IM	2	50µl	Each quadricep
Guinea-pig	IM	2	50µl	Each quadriceps
	SC	4	50µl	On each shoulder and each quadricep
Rabbit	IM	2	40µl	Each quadriceps On each shoulder and each quadricep
		4	100µl	
	ID	10	10µl	Along the neck
Goat/Sheep	IM	4	250µl	2 injections in each quadriceps
Chicken/Turkey	IM	2	50µl	In each pectoral muscle
	SC	1	100µl	In the neck
Horse/Cow	SC	10	100µl	Along the neck

Some good responses were obtained with a relation water/TiterMax ranging from 10/90 to 90/10 but the relation 50/50 is usually optimal

IM : Intramuscular SC : Subcutaneous ID : IntraDermal

Related products and documents

page [B91+](#) Monoclonal antibody production, i.e. Hybridokine [UP826430](#) (hybridoma enhancing growth factor)
 page [B17+](#) Antigen preparation, i.e. MaxiBind carriers [UP84225A](#) (BSA, OVA and bLG with 100% increased coupling capacity)
 page [B166+](#) Crosslinkers
 Antibody Purification

Ordering information

Catalog size quantities and prices may be found at <http://www.interchim.com>

Please inquire for higher quantities (availability, shipment conditions).

For any information, please contact [uptima](#) – Interchim for any question / Interchim; Hotline : +33(0)4 70 03 73 06

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