

# AddaVax™

Squalene-based oil-in-water adjuvant

Catalog code: vac-adx-10

<https://www.invivogen.com/addavax>

For research use only. Not for use in humans.

Version 23F19-AK

## PRODUCT INFORMATION

### Contents

- 10 ml of AddaVax™ provided as a ready-to-use sterile emulsion

### Storage and stability

- AddaVax™ is shipped at room temperature.
- Store at 4°C. **DO NOT FREEZE**. Do **not** store in plastic tubes.
- AddaVax™ is stable for 2 years from the date of manufacture (see certificate of analysis for further information).

### Formulation

AddaVax™ is based on nano-emulsification of 2 components:

- Sorbitan trioleate (0.5% w/v) in squalene oil (5% v/v)
- Tween 80 (0.5% w/v) in sodium citrate buffer (10 mM, pH 6.5)

The nano-emulsion is produced using a microfluidizer and filtered through a 0.22-µm filter to remove large droplets and sterilize the final product. The particle size is ~ 160 nm.

### Quality control

- AddaVax™ is VacciGrade™ (preclinical grade). It is prepared under strict aseptic conditions and is tested for the presence of endotoxins. AddaVax™ is guaranteed sterile and its endotoxin level is < 1 EU/ml (measurement by kinetic chromogenic LAL assay).
- Adjuvanticity of AddaVax™ was evaluated by assessing the levels of total mouse IgG (mIgG) and the mIgG1 and mIgG2 isotypes after two subcutaneous injections of EndoFit™ Ovalbumin/AddaVax™ (1:1, v/v) in mice. Results were compared to mice receiving antigen alone.

## DESCRIPTION

AddaVax™ is a squalene-based oil-in-water nano-emulsion based on the formulation of MF59® that has been licensed in Europe for adjuvanted flu vaccines<sup>1</sup>. Squalene is an oil that is more readily metabolized than the paraffin oil used in Freund's adjuvants<sup>2</sup>. Squalene oil-in-water emulsions, such as MF59®, elicit both cellular (Th1) and humoral (Th2) immune responses<sup>2,3</sup>. This class of adjuvants is believed to act through a depot effect, enhancement of antigen persistence at the injection site, recruitment and activation of antigen presenting cells, and direct stimulation of cytokine and chemokine production by macrophages and granulocytes<sup>1</sup>. Typical results obtained with AddaVax™ are shown in Figure 1.

MF59® is a registered trademark used for adjuvants for vaccine owned by Novartis Ag.

1. Mbow ML. *et al.*, 2010. New adjuvants for human vaccines. *Curr Opin Immunol* 22(3):411-6. 2. Calabro, S. *et al.*, 2013. The adjuvant effect of MF59 is due to the oil-in-water emulsion formulation, none of the individual components induce a comparable adjuvant effect. *Vaccine* 31:3363-9. 3. Ott G. *et al.*, 1995. MF59. Design and evaluation of a safe and potent adjuvant for human vaccines. *Pharm Biotechnol* 6:277-96.

## METHODS

### Preparation of antigen-AddaVax™ mixture

Antigens are preferentially diluted in saline or phosphate buffers. The amount of protein or conjugated peptide used for the primary immunization can be adjusted depending upon availability and immunogenicity of the antigen. Mice can be injected subcutaneously (s.c.) with 1 to 10 µg of endotoxin-free ovalbumin (cat. code vac-pova).

1. Bring AddaVax™ to room temperature.
2. Shake the capped bottle of AddaVax™ before opening.
3. Mix equal volumes of antigen and AddaVax™ by pipetting.

The volume of injection depends of the site of administration. For example, 100 µl can be injected s.c. in mice.

*Note:* To avoid anaphylaxis, do not use adjuvants for intravenous injection.

Recommended maximum volumes for injection of antigen/adjuvant mixtures per site of injection for laboratory animals. (Lindblad EB., 2000. Freund's Adjuvants. In: Vaccine adjuvants: Preparation Methods and Research Protocols. Humana Press. Totowa, NJ).

Species	Max. volume	Injection Site
Mice, hamsters	100 µl	subcutaneous (s.c.)
Mice, hamsters	50 µl	intramuscular (i.m.)
Guinea pigs	200 µl	s.c. or i.m.
Rats	200 µl	s.c. or i.m.
Rabbits	250 µl	s.c. or i.m.

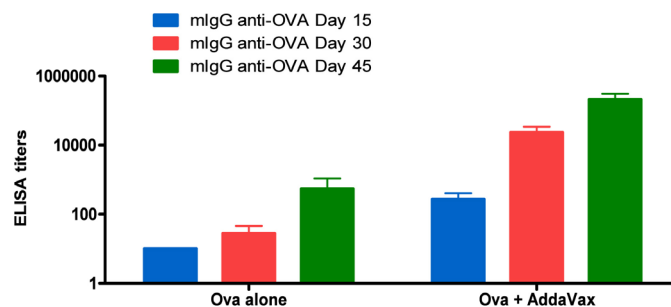


Figure 1. Anti-Ova mIgG levels at 15, 30 and 45 days after the initial immunization in different groups. Mice were immunized s.c. at 0, 2 and 3 weeks with 1 µg of EndoFit™ Ovalbumin alone or 1 µg of EndoFit™ Ovalbumin/AddaVax™ (1:1, v/v) in a final volume of 100 µl. Serum anti-OVA mIgG was monitored by ELISA (coated with ovalbumin at 10 µg/ml in PBS).

## TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Asia: +852 3622-34-80

E-mail: [info@invivogen.com](mailto:info@invivogen.com)

## RELATED PRODUCTS

Product	Description	Catalog Code
<b>Vaccine Adjuvants</b>		
Alhydrogel 2%	Aluminium hydroxide gel	vac-alu-250
CFA	Complete Freund's adjuvant	vac-cfa-10
IFA	Incomplete Freund's adjuvant	vac-ifa-10
Poly(I:C) HMW VacciGrade™	TLR3 agonist	vac-pic
MPLA-SM VacciGrade™	TLR4 agonist	vac-mpla2
Flagellin FliC VacciGrade™	TLR5 agonist	vac-fla
Imiquimod VacciGrade™	TLR7 agonist	vac-imq
R848 VacciGrade™	TLR7/8 agonist	vac-r848
ODN 1826 VacciGrade™	Murine TLR9 agonist	vac-1826-1
ODN 2006 VacciGrade™	Human TLR9 agonist	vac-2006-1
<b>OVA Antigens</b>		
EndoFit™ Ovalbumin (endotoxin-free)	For <i>in vivo</i> use; endotoxin level <1 EU/mg	vac-pova
Ovalbumin	For detection; Western, ELISA	vac-stova
Ova 257-264	For detection; ELISPOT	vac-sin
Ova 323-339	For detection; ELISPOT	vac-isq

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