

# SqualVax

## Squalene based adjuvant

### Product information:

**SqualVax** is a squalene oil-in-water emulsion similar to MF59®. **SqualVax** is available in three quantities: #SQ0010: 10 mL, #SQ0050: 5x10 mL & #SQ100: 10x10 mL.

### Storage and stability:

Shipping and storage: SqualVax is shipped at room temperature and stored at +4°C. SqualVax is stable for 6 months. DO NOT FREEZE.

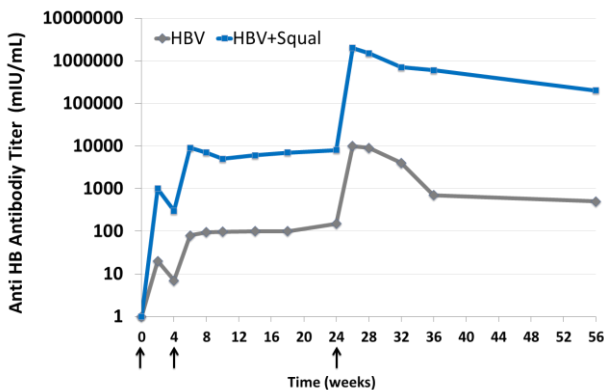
### Description.

SqualVax is an oil-in-water emulsion made of squalene droplets in a continuous aqueous phase. It is fully biodegradable, which is an important advantage over alternative oils that have been used in emulsion adjuvants, like Freund's adjuvant that contains mineral oil (paraffin oil) and has long term persistence in the organisms.

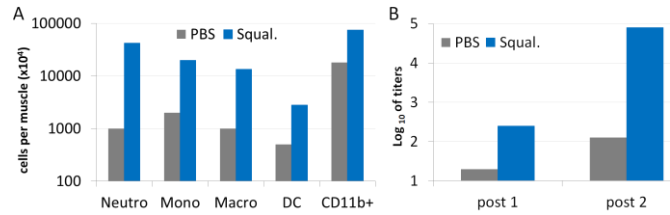
Squalene emulsion induces local stimulation and recruitment of DCs and granulocytes, differentiation of monocytes into DCs and increased uptake of antigen by DCs. The emulsion acts more specifically on macrophages present at the site of injection. A local increase of chemokines released also influences the recruitment of immune cells from the blood to the site of vaccination, creating an amplification loop. This formulation enhances differentiation of monocytes towards a mature phenotype, thereby promoting migration of antigen-loaded cells to the draining lymph node. Compared to aluminium salts, a stronger immune response is elicited (e.g higher antibody "humoral response, Th2" and T-cell response "cellular response Th1") with a mixed and more balanced Th1/Th2 cell phenotype.

### Results

Results presented below demonstrate the effect of squalene emulsion adjuvant on immune system response:



**Figure 1.** Duration of antibody response in baboons vaccinated against hepatitis B virus (HBV) at weeks 0, 4 and 24 with or without squalene emulsion. Adapted from Traquina P. *et al.*, *J Infect Dis.* 1996; 174(6):1168-75.



**Figure 2. Innate and adaptive immune responses in mice after squalene-adjuvanted immunization.** (A) Cell recruitment in muscle 24H after injection (IM) of ovalbumine (OVA; 10 µg per mouse) in presence of PBS or squalene emulsion. (B) OVA-specific antibody titers enhanced by squalene emulsion. Mice were immunized twice with OVA in PBS or squalene emulsion and OVA-specific antibody titers were measured by ELISA after the first and second immunization (adapted from Vono M. *et al.*, *PNAS.* 2013; 110(52):21095-100).

### Method/protocol.

#### Recommendations before starting:

The inoculum should be free of extraneous microbial contamination; filtration of the antigen before mixing with the adjuvant is recommended. Allow SqualVax reagent to reach room temperature before beginning.

1. Vigorously shake the SqualVax vial before opening
2. Dilute antigen mixture in saline buffer or phosphate buffer for a final immunogen concentration of 10-100 µg/100 µL
3. Mix SqualVax adjuvant with an equal volume of antigen solution for a 1:1 ratio
4. Pipette up and down several times to ensure correct mix
5. Inject into the animal according to the table below; the volume depends on the site of injection. Typical routes of administration include subcutaneous (SC), intramuscular (IM), intradermal (ID) or intraperitoneal (IP).

Species	Max vol/site	Primary injection	Subsequent Injection(s)
Mice, hamsters	0.1 mL	SC	SC
Mice, hamsters	0.05 mL	IM <sup>A</sup>	IM <sup>A</sup>
Mice	0.5 mL	IP <sup>x</sup>	SC, IM <sup>A</sup>
Guinea pigs, rats	0.2 mL	SC, IM <sup>A</sup>	SC, IM <sup>A</sup>
Rabbits	0.25 mL	SC, IM	SC, IM
Rabbits	0.025 mL	ID	SC, IM
Sheep, goats, donkeys, pigs	0.5 mL	SC, IM	
Chickens			

<sup>A</sup> Not recommended in general, in particular not for viscous adjuvants

<sup>x</sup> Not recommended for pAb production

**Table 1:** Maximum volumes for injection of immunogen/adjuvant mixtures per site of injection for different animal species (Adapted from Leenars MPPA, Hendriksen CFM *et al.*, 1999).

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