

### Content and product information

**CaLiVax-DOTAP** is a cationic lipid-based composition for liposome-mediated DNA or protein vaccine. This adjuvant has an average size of 100 nm.

### Description

**DOTAP** (N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N trimethyl-ammonium methyl-sulfate):Cholesterol (1:1 molar ratio) cationic lipid composing this genetic adjuvant allows the formation of complexes with plasmid DNA or antigen protein to form an efficient liposome-based nanoparticle delivery system (LPD). LPD are **non-viral gene delivery systems**, self-assembled from cationic liposomes and negatively charged immunogen that function as **vaccine carrier**. DOTAP cationic liposome adjuvant is compatible with most immunization procedures: such as intramuscular, intraepidermal, intravenous, intraperitoneal or subcutaneous.

### Use, handling and storage

*For Research Use Only. Not for use in humans. Not for use in diagnostic or therapeutic purposes.*

**Shipping conditions:** Room Temperature.

**Storage conditions:** 4°C.

**Shelf life:** 1 year from the date of purchase .

⚠ Do not freeze.

### Kit contents

**CV02000:** 2x1 mL of CaLiVax-DOTAP.

Certificate of analysis on demand.

### 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.

1. Allow CaLiVax adjuvant and immunogen solution to reach room temperature before beginning.
2. Vigorously shake the CaLiVax vial before opening.
3. Dilute immunogen in saline buffer or phosphate buffer for a final immunogen concentration of **2-50 µg/50 µL**. It is mandatory to not use buffers containing serum.
4. Mix CaLiVax adjuvant with an equal volume of immunogen solution for a **1:1** ratio to obtain a N/P ratio equals to **1.25** to **25**, respectively.
5. Pipette up and down several times to ensure correct mix.
6. Incubate at room temperature for **20-30 min**.
7. Inject into the animal according to the table below. Repeat injection **3 times, 2 weeks** apart to ensure the maximum of immune answer.

NOTE: do not store the complexes: discard solution after use. Prepare fresh LPD before each immunization.

Volume of injection depends on the site of injection and the animal model.

Typical routes of administration include subcutaneous (SC), intramuscular (IM), intradermal (ID) or intraperitoneal (IP).

Species	IM	SC	ID	IP
Mice, hamsters	0.05-0.1 mL	0.1-0.2 mL	0.025 mL	0.5 mL
Guinea pigs, rats	0.1-0.2 mL	0.2-0.4 mL	0.025 mL	1.0 mL
Rabbits	0.25 mL	0.25 mL	0.025 mL	10 mL
Pigs	0.25-0.5 mL	0.5 mL	0.5 mL	50 mL

**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., 19).

### Purchaser Notification | Conditions of Sale

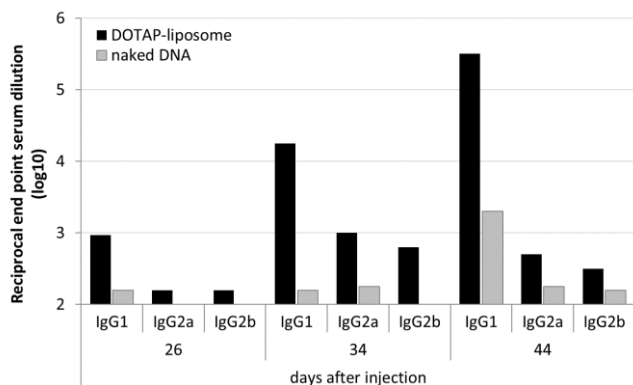
This product is sold in accordance with our general conditions of sale that you can find on our website: <https://ozbiosciences.com/content/3-terms-and-conditions>.

## Plasmid DNA

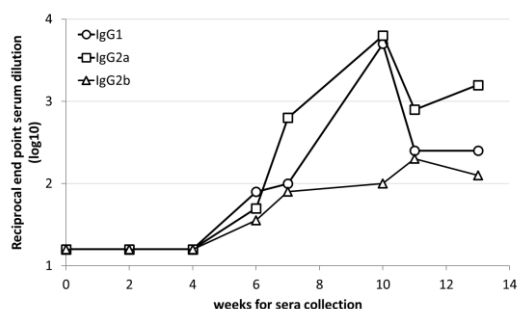
Cationic liposome-mediated antigen-coding plasmid DNA has been shown to greatly improve humoral and cell-mediated immunity. One of the possibilities is that these DNA vaccines could facilitate uptake of the plasmid by antigen-presenting cells (APC) and induce cytotoxic T lymphocyte response. Moreover, once entrapped into lipoplexes, DNA is protected from nucleases and depending on their size, some lipoplexes may break down locally to release their vaccine content slowly; the accessibility of genetic material is thus prolonged. State of the art studies report that BALB/C mice injected repeatedly by the intramuscular route with low doses of plasmid entrapped in DOTAP cationic liposomes elicited up to a 100-fold antibody (IgG1) response than animals immunized with naked DNA alone.

## Results

Results presented below demonstrate the effect of DOTAP-liposomes adjuvant on immune system response:



**Figure 1. Comparison of immune response in mice injected with naked or DOTAP-liposome entrapped DNA.** Sera were tested at the indicated days post intramuscular injection and analyzed by ELISA (adapted from Chen WC., *Adv Genet.* 2005; 54:315-37).



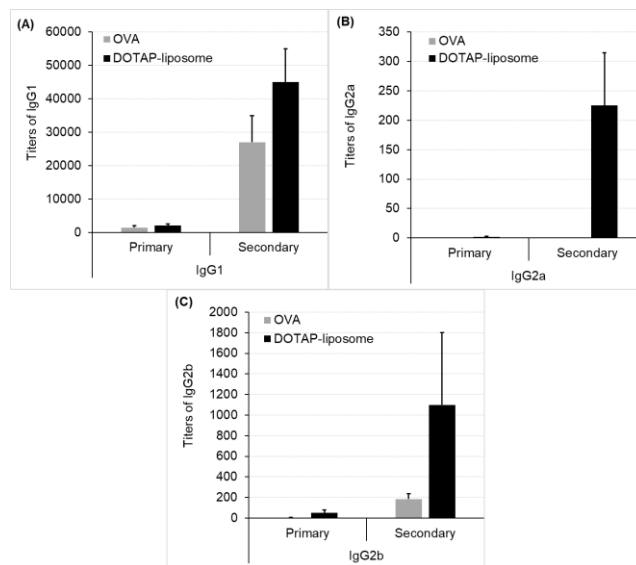
**Figure 2. Time course of immune responses in mice immunized with plasmid entrapped in DOTAP-liposome.** Sera samples were collected at various time intervals and tested by ELISA for IgG1 (circles), IgG2a (squares) and IgG2b (triangles) (adapted from Perrie Y., *Vaccine.* 2001 Apr 30;19(23-24):3301-10).

## Protein antigen

The study reports that DOTAP liposomes dramatically improved the production of secondary anti-OVA IgG2a and IgG2b, highlighting its adjuvanting capacity (Fig.3). The enhanced secondary anti-OVA immune responses are due to the immunostimulatory effect of DOTAP liposome, including promoting DC maturation.

## Results

Results presented below demonstrate the effect of DOTAP liposomes-mediated protein vaccine on immune system response:



**Figure 3. The effect of liposomes on OVA-specific immune responses.** Six-week-old female C57BL/c mice were s.c. immunized with free OVA (25µg/mouse) or liposome encapsulated OVA. Primary and secondary anti-OVA IgG1 (A), IgG2a (B) and IgG2b (C) in plasma were measured using ELISA (adapted from Zhuang Y., *J. Control. Release.* 2012; 159, 135-142).

## Related Products

Ref	Description
#AH0250	AlumVax Hydroxide 2%
#AP0250	AlumVax Phosphate 2%
#SQ0100	SqualVax, squalene oil-in-water emulsion
#IFA0100	Incomplete Freund's Adjuvant (IFA)
#CFA0100	Complete Freund's Adjuvant (CFA)
#LV02000	LipoVax NTA(Ni)