

## CaLiVax-DOTAP Vaccine Adjuvant Cationic Liposome-DOTAP

### 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.

**CaLiVax-DOTAP** is available in one quantity – 2mL.  
#CV02000 2x1mL.

### Storage and stability

Shipping and storage: CaLiVax is shipped at RT and stored at +4°C.

CaLiVax is stable for 1 year. DO NOT FREEZE.

### 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.

### Method/protocol

#### Recommendations before starting:

The inoculum should be free of extraneous microbial contamination; use plasmid DNA or Protein antigen as pure as possible. Adapt volumes according to the table 1 below.

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 buffer containing serum.
4. Mix CaLiVax adjuvant with an equal volume of immunogen solution for a 1:1 ratio in order 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 (mL) for injection depends on the site of injection and the animal model. Typical routes of administration include intramuscular (IM), subcutaneous (SC), intradermal (ID) or intraperitoneal (IP).

| Species           | I.M.     | S.C.    | I.D.  | I.P |
|-------------------|----------|---------|-------|-----|
| Mice, hamsters    | 0.05-0.1 | 0.1-0.2 | 0.025 | 0.5 |
| Guinea pigs, rats | 0.1-0.2  | 0.2-0.4 | 0.025 | 1.0 |
| Rabbits           | 0.25     | 0.25    | 0.025 | 10  |
| Pigs              | 0.25-0.5 | 0.5     | 0.5   | 50  |

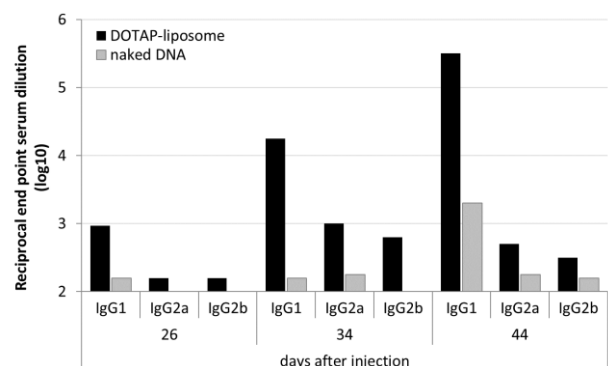
**Table 1:** Recommended volumes for injection of immunogen/adjuvant mixtures per site of injection for different animal species (Adapted from Leenars MPPA, Hendriksen CFM et al., 1999).

#### • Plasmid DNA

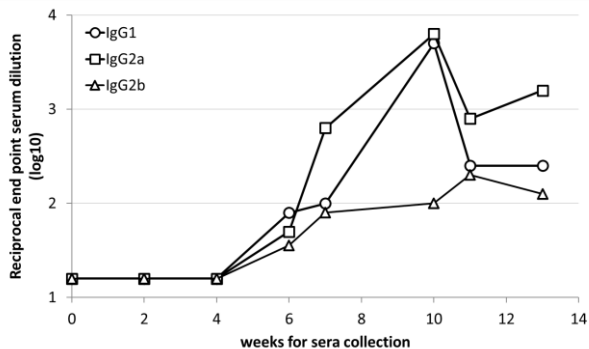
Cationic liposome-mediated antigen-coding plasmid DNA has been shown to greatly improve humoral and cell-mediated immunity. One of the possibilities is that these DNA vaccines could facilitate uptake of the plasmid by antigen-presenting cells (APC) and induce cytotoxic T lymphocyte response. Moreover, 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.

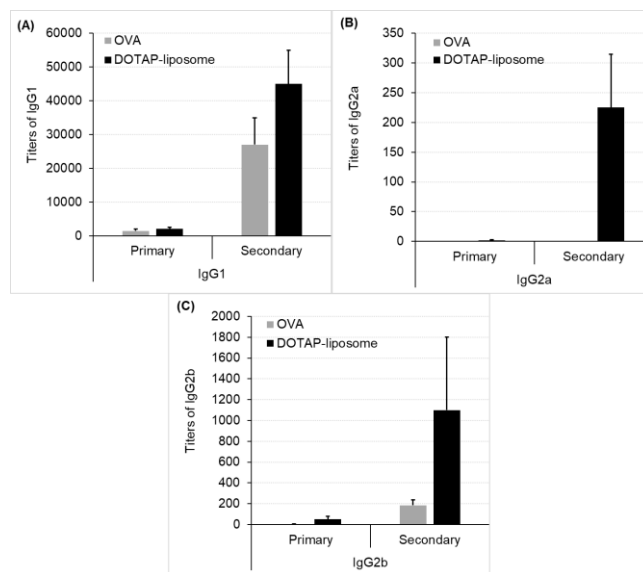


**Figure 2. Time course of immune responses in mice immunized with plasmid entrapped in DOTAP-liposome.** Sera samples were collected at various time intervals and tested by ELISA for IgG1 (circles), IgG2a (squares) and IgG2b (triangles). Adapted from Perrie Y., *Vaccine*, 2001 Apr 30;19(23-24):3301-10.

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



**Fig 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.

**Purchaser Notification**

**Limited License**

The purchase of the CaliVax Vaccine Adjuvant grants the purchaser a non-transferable, non-exclusive license to use the included components. This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this formulation is excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the CaliVax Vaccine Adjuvant. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact the Director of Business Development at OZ Biosciences.

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**Product Use Limitations**

The CaliVax Vaccine Adjuvant is developed, designed, intended, and sold for research use only. It is not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the component by following proper research laboratory practices.

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