

# LipoVax NTA(Ni) Vaccine Adjuvant His-Tagged-based Protein or Peptide Carrier Vaccine Adjuvant

# **Product information**

**LipoVax NTA(Ni)** is Ni<sup>2+</sup>-based liposome adjuvant that can anchor diverse histidine-tagged proteins or peptides to an antigen-presenting carrier to trigger immune response (immunization, vaccination, antibody generation).

**LipoVax NTA(Ni)** is available in one quantity – 2mL; #LV02000 2x1mL.

### Storage and stability

Shipping and storage: LipoVax is shipped at RT and stored at +4°C. LipoVax is stable for 6 months for unopened vials stored at 4°C. DO NOT FREEZE.

### Description

**LipoVax NTA(Ni)** is a liposome-based adjuvant containing phospholipid, cholesterol and nickel-chelating lipid (60:39:1), showing high affinity to bond with electron-rich ligand such as histidine. Therefore, complexes can be generated using Ninitrilotriacetic acid (NTA) and his-tagged proteins or peptides. The resulting liposomes are **non-viral biologics delivery systems**, self-assembled from metal-chelating lipid and his-tagged immunogens such as envelope glycoproteins. Nickel-based liposome adjuvant is compatible with most immunization procedures: such as <u>intramuscular</u>, <u>intraepidermal</u>, <u>intravenous</u>, intraperitoneal or subcutaneous.

### Method/protocol

# Recommendations before starting:

The ligand or protein antigen should be free of extraneous microbial contamination.

- 1. Allow LipoVax NTA(Ni) adjuvant solution to reach room temperature before beginning.
- 2. We recommend to dilute antigen mixture in saline buffer or phosphate buffer for a final immunogen concentration of 10-100  $\mu$ g/100  $\mu$ L\* and incubate 1h-2h at RT.
- 3. Mix LipoVax adjuvant with an equal volume of antigen solution for a 1:1 volume ratio.
- 4. For the removal of the non-conjugated ligand, we recommend to proceed by gel filtration or by dialysis. For the dialysis use 1L PBS; operate with MWCO below 1,000 kDa. Quantify the protein association with Protein Assay.
- 5. Inject into the animal according to the table below.

\* For record, the total lipid concentration is 11.25 mM comprising 1 %mol of NTA(Ni) lipid. Only half is exposed to the liposome surface, which equals to 55 nM of conjugable lipid.

Note: Ratio can be optimized from 1:1 to 1:9 (100µL adjuvant per 900µL antigen). Do not store the complexes.

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 immunogenadjuvant mixtures per site of injection for different animal species (Adapted from Leenars MPPA, Hendriksen CFM et al., 1999).

#### • Envelope protein antigen nanocarrier

A major step toward in vaccination field is an immunogen capable of eliciting neutralizing antibodies. Liposomal nanoparticles platform that presents well-ordered viral trimeric proteins (e.g. gp140 HIV-1) were superior to vehicle proteins for induction of antigen-specific antibody responses. It has been shown that such liposomes promote dendritic cells maturation and elicit long-lasting neutralizing antibodies responses. The antigen-conjugated liposomes are a promising initial lead for the development of new vaccines.

### Results

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

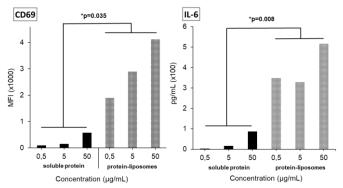


Figure 1. Activation of primary B cells by soluble protein and proteinconjugated liposomes. CD69 cell surface activator was analyzed by flow cytometry and IL-6 level secreted in B cells supernatant was measured by ELISA. Adapted from Wyatt R. T., *et al.*, *Cell Reports*, 2016; 15(9): 1986-1999.

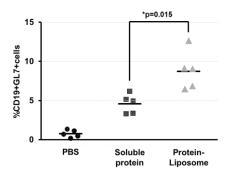


Figure 2. S.c. immunization of C57BI/6 mice with protein-anchored liposomes induced enhanced germinal center formation. After 14 days, lympho node B cells were analyzed for the activation marker, GL7. The percentages of CD19+ GL7+ cells are enumerated. Adapted from Wyatt R. T., et al., Cell Reports, 2016; 15(9): 1986-1999.

OZ Biosciences – the Art of Delivery Systems www.ozbiosciences.com | tech@ozbiosciences.com

### **References and background reading**

- Platt, V., Huang, Z., et. al. (2010). Influence of multivalent nitrilotriacetic acid lipid– ligand affinity on the circulation half-life in mice of a liposome-attached his6protein. Bioconjugate chemistry, 21(5), 892-902.
- Pejawar-Gaddy, S., Kovacs, J. M., Barouch, D. H., Chen, B., & Irvine, D. J. (2014). Design of lipid nanocapsule delivery vehicles for multivalent display of recombinant Env trimers in HIV vaccination. *Bioconjugate chemistry*, 25(8), 1470-1478.
- Ingale, J., Stano, A., Guenaga, J., Sharma, S. K., Nemazee, D., Zwick, M. B., & Wyatt, R. T. (2016). High-density array of well-ordered HIV-1 spikes on synthetic liposomal nanoparticles efficiently activate B cells. *Cell reports*, 15(9), 1986-1999.
- Cale, E. M., Gorman, J., Radakovich, N. A., Crooks, E. T., Osawa, K., Tong, T., & Binley, J. M. (2017). Virus-like particles identify an HIV V1V2 apex-binding neutralizing antibody that lacks a protruding loop. *Immunity*, 46(5), 777-791.
- Bale, S., Goebrecht, G., Stano, A., Wilson, R., Ota, T., Tran, K. & Wyatt, R. T. (2017). Covalent linkage of HIV-1 trimers to synthetic liposomes elicits improved B cell and antibody responses. Journal of virology, 91(16), e00443-17.

#### **Purchaser Notification**

#### **Limited License**

The purchase of the LipoVax NTA(Ni) 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 LipoVax NTA(Ni) 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.

Buyers may end this License at any time by returning all LipoVax NTA(Ni) Vaccine Adjuvant material and documentation to OZ Biosciences, or

#### **OZ Biosciences SAS**

163 avenue de Luminy Case 922, zone entreprise 13288 Marseille cedex 09 - FRANCE Ph: +33 (0) 486 948 516 Fax: +33 (0) 486 948 515 contact@ozbiosciences.com order@ozbiosciences.com

Rev 01/22AB

- Pauthner, M., Havenar-Daughton, C., Sok, D., Nkolola, J. P., Bastidas, R., Boopathy, A. V. & Burton, D. R. (2017). Elicitation of robust tier 2 neutralizing antibody responses in nonhuman primates by HIV envelope trimer immunization using optimized approaches. *Immunity*, 46(6), 1073-1088.
- Martinez-Murillo, P., Tran, K., Guenaga, J., Lindgren, G., Àdori, M., Feng, Y. & Hedestam, G. B. K. (2017). Particulate array of well-ordered HIV clade C Env trimers elicits neutralizing antibodies that display a unique V2 cap approach. Immunity, 46(5), 804-817.
- Dubrovskaya, V., Tran, K., Ozorowski, G., Guenaga, J., Wilson, R., Bale, S. & Wyatt, R. T. (2019). Vaccination with glycan-modified HIV NFL envelope trimer-liposomes elicits broadly neutralizing antibodies to multiple sites of vulnerability. *Immunity*, *51*(5), 915-929.
- Thalhauser, S., Peterhoff, D., Wagner, R., & Breunig, M. (2020). Critical design criteria for engineering a nanoparticulate HIV-1 vaccine. *Journal of Controlled Release*, 317, 322-335.

by destroying LipoVax NTA(Ni) Vaccine Adjuvant components. Purchasers are advised to contact OZ Biosciences with the notification that a LipoVax NTA(Ni) Vaccine Adjuvant is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s).

This document covers entirely the terms of the LipoVax NTA(Ni) Vaccine Adjuvant research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

#### **Product Use Limitations**

The LipoVax NTA(Ni) 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.

#### **OZ Biosciences INC**

4901 Morena Blvd, Suite 901 San Diego CA 92117 - USA Ph: + 1-858-246-7840 Fax: + 1-855-631-0626 contactUSA@ozbiosciences.com orderUSA@ozbiosciences.com