

AlumVax Hydroxide 2% Vaccine Adjuvant

Product information and content

AlumVax Hydroxide is wet gel (colloidal) of aluminum hydroxide 2%, provided as a ready-to-use suspension. It is sterilized and aseptically filled. AlumVax hydroxide is a crystalline aluminum oxyhydroxide that is positively charged at physiological pH (pI=11), suitable for adsorption of negatively charged, acidic proteins (such as albumin). **AlumVax Hydroxide** is available in two quantities:

#AH0050: 50 mL and #AH0250: 250 mL.

Storage and stability.

<u>Shipping and storage:</u> Room Temperature. Product is stable for 6 months. DO NOT FREEZE (ice crystal may impair the product).

Chemical properties.

CAS Number: 21645-51-2

Formulation: Al(OH)3, Aluminium hydroxide gel Appearance: White gelatinous precipitate Aluminium content: 9.0 – 11.0 mg/ml; pH: ~6.5

Description.

Aluminum hydroxide is the most common adjuvant used in approved prophylactic vaccines because of its excellent safety profile and ability to enhance protective humoral immune response. Since more than 80 years, it has been observed that aluminium compounds act by a depot effect and also by direct activation of the immune cells. Adsorption or entrapment of antigens in aggregates through hydrophobic and electrostatic interactions favors a high local antigen concentration and improved uptake by antigen presentating cells (APC).

AlumVax hydroxide stimulates Th2 response through the release of Th2-associated cytokines (IL4, IL-5, IL-13...) and Th2-associated antibodies (IgG1 & IgE). It increases Agspecific CD4⁺ T Cell proliferation and promotes NALP3 inflammasome activation and caspase 1-mediated release of IL-1 and IL-18.

NOTE: Alum is frequently used as an alternative to Freund's adjuvants, as it is less hazardous and less likely to cause tissue necrosis at the injection site.

Results.

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

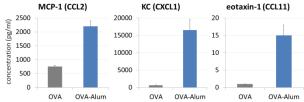


Figure 1. Response of innate immune system cells to injection of Ag +/- Alum Adjuvant. The injection of ovalbumin (OVA) adsorbed onto Alum (OVA-Alum) in mice induces a marked increase in the levels of the monocyte chemotactic protein (MCP1; CCL2), the neutrophil chemotaxin KC (CXCL1) and the eosinophil chemotaxin eotaxin-1 (CCL11) compared to mice receiving OVA alone (adapted from Kool M. et al., J Exp Med. 2008; 205(4):869-82).

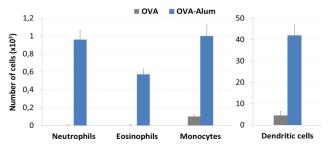


Figure 2. Innate inflammatory response induced by alum. Mice were injected i.p. with ovalbumin (OVA) or OVA-Aluminum hydroxide. 24H later, number of Neutrophils, Eosinophils and Monocytes and dendritic cells was determined in peritoneal lavage by flow cytometry (adapted from Kool M. *et al.*, J Immunol. 2008; 181(6):3755-9).

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.

Preparation of AlumVax hydroxide-immunogens mix is easier than other adjuvants as it does not require laborious emulsification.

- 1. Ensure complete re-suspension of AlumVax hydroxide adjuvant by vigorous shaking of the vial before use.
- 2. Dilute antigen mixture in saline buffer or phosphate buffer for a final immunogen concentration of 10-100 $\mu g/100~\mu L$.
- 3. Mix AlumVax hydroxide adjuvant with an equal volume of antigen solution for a 1:1 ratio:
 - a. Add AlumVax hydroxide dropwise with constant mixing to the immunogen solution.
 - b. Pipet up and down several times to ensure correct absorption of antigen by alum adjuvant and incubate 5 to 10 minutes.

NOTE: Ratio can be optimized from 1:1 (100 μ L adjuvant per 100 μ L antigen) to 1:9 (100 μ L adjuvant per 900 μ L antigen)

 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^Δ	IM^Δ
Mice	0.5 mL	IP^{\times}	SC, IM [∆]
Guinea pigs, rats	0.2 mL	SC, IM [∆]	SC, IM [∆]
Rabbits	0.25 mL	SC, IM	SC, IM
Rabbits	0.025 mL	ID	SC, IM
Sheep, goats, donkeys, pigs Chickens	0.5 mL	SC, IM	

 $^{^{\}Delta}$ Not recommended in general, in particular not for viscous adjuvants

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

 $^{^{\}times}$ Not recommended for pAb production