

# Alhydrogel® adjuvant 2%

Aluminium hydroxide gel

Catalog # vac-alu-250

<http://www.invivogen.com/alhydrogel>

Version # 17C21-MM

## PRODUCT INFORMATION

### Content:

250 ml of Alhydrogel® adjuvant 2% is provided as a ready-to-use, aluminium hydroxide wet gel (colloidal) suspension. Alhydrogel® adjuvant is sterilized by autoclavation and aseptically filled.

### Storage and stability:

- Alhydrogel® adjuvant 2% is shipped at room temperature and should be stored at room temperature. The expiry date is specified on the product label. **DO NOT FREEZE.**

*Note: Do not expose to frost as product will be destroyed if ice crystals form in the gel.*

### Quality control:

- Alhydrogel® adjuvant 2% is tested for pyrogenicity and sterility.

## CHEMICAL PROPERTIES

**CAS Number:** 21645-51-2

**Formulation:** Al(OH)<sub>3</sub>, Aluminium hydroxide gel

**Appearance:** White gelatinous precipitate

**Aluminium content:** 9.0 – 11.0 mg/ml

**pH:** ~6.5

## DESCRIPTION

Alhydrogel® adjuvant is an aluminium hydroxide (referred to as alum) wet gel suspension. Alum improves attraction and uptake of antigen by antigen presenting cells (APCs)<sup>1</sup>. Recently, it has been suggested that the antigens adsorbed on the aluminium salts are presented in a particulate form, making them more efficiently internalized by APCs. Moreover, alum activates the NLRP3 inflammasome complex implicated in the induction of several pro-inflammatory cytokines including IL-1 $\beta$  and IL-18<sup>2</sup>. Alum increases Th2 antibodies but does not promote significant Th1 cellular response<sup>3</sup>. Alhydrogel® particles have a net positive electrical charge at pH 5-7 and thus are well suited for adsorption of negatively charged antigens (e.g. antigens with isoelectric points below the pH of formulation)<sup>4</sup>. Alhydrogel® adjuvant 2% is made by Brenntag Biosector, a leader in the global vaccine adjuvants market with a long history of producing high quality products. Alhydrogel® adjuvant 2% was elected as the International Standard Preparation for aluminium hydroxide gels<sup>4, 5</sup>. Alhydrogel® adjuvant 2% is present in multiple commercial vaccine formulations<sup>2, 6</sup>. Typical results obtained with Alhydrogel® adjuvant 2% are shown in figure 1.

1. **Coffman R. et al., 2010.** Vaccine adjuvants: Putting innate immunity to work. *Immunity* 33(4):492-503. 2. **Marrack P. et al., 2009.** Towards an understanding of adjuvant action of aluminium. *Nat Rev Immunol.* 9(4): 287-93. 3. **Huang M. & Wang W., 2014.** Factors affecting alum-protein interactions. *Int J Pharm.* 466(1-2):139-46. 4. **Stewart-Tull D., 1989.** Recommendations for the assessment of adjuvants (immunopotentiators). In: *Immunological adjuvants and vaccines* (Gregoriadis, G., Allison, A. & Poste, G., eds.), Plenum, New York, pp. 213–226. 5. **Stewart-Tull, D., 1991.** The assessment and use of adjuvants. In: *Vaccines*. (Gregoriadis, G., Allison, A., & Poste, G., eds.), Plenum, New York, pp.85–92. 6. **Lindblad E. & Schonberg N., 2010.** Aluminum adjuvants: preparation, application, dosage, and formulation with antigen. *Methods Mol Biol.* 626:41-58.

## METHODS

### Preparation of antigen-Alhydrogel® adjuvant 2% mixture

Antigens are preferentially diluted in saline or phosphate buffers. The amount of protein or conjugated peptide used for the primary immunization can be adjusted depending upon availability and immunogenicity of the antigen. For example, mice can be injected subcutaneously (s.c.) with 1 to 10  $\mu$ g of endotoxin-free ovalbumin (cat. code #vac-pova). The adsorption capacity for a model protein such as diphtheroid toxoid, human growth hormone or ovalbumin in Alhydrogel® adjuvant varies from 1 to 3 mg (mg/mg Al)<sup>3</sup>.

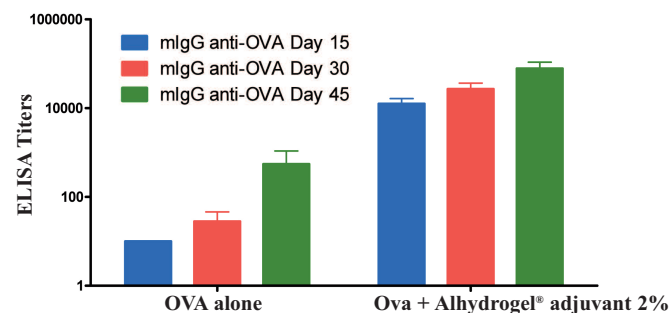
1. Shake well the capped bottle of Alhydrogel® adjuvant 2% before use.
2. Add Alhydrogel® adjuvant 2% to the antigen solution; the final volume ratio of Alhydrogel® adjuvant 2% to antigen should be 1:1 (100  $\mu$ l Alhydrogel® adjuvant 2% for 100  $\mu$ l of antigen) to 1:9 (100  $\mu$ l Alhydrogel® adjuvant 2% for 900  $\mu$ l of antigen).
3. Mix well by pipetting up and down for at least 5 minutes to allow Alhydrogel® adjuvant 2% to effectively adsorb the antigen.

The volume of injection depends on the site of administration. For example, 100-200  $\mu$ l can be injected s.c. in mice.

*Note: To avoid anaphylaxis, do not use adjuvants for intravenous injection.*

### Recommended maximum volumes for injection of antigen/adjuvant mixtures per site of injection for laboratory animals.

Species	Max. volume	Injection Site
Mice, hamsters	100 $\mu$ l	subcutaneous (s.c.)
Mice, hamsters	50 $\mu$ l	intramuscular (i.m.)
Guinea pigs	200 $\mu$ l	s.c. or i.m.
Rats	200 $\mu$ l	s.c. or i.m.
Rabbits	250 $\mu$ l	s.c. or i.m.



**Figure 1.** Anti-Ova mIgG levels at 15, 30 and 45 days after the initial immunization in different groups. Mice were immunized s.c. at 0, 2 and 3 weeks with 1  $\mu$ g of EndoFit™ Ovalbumin alone or 1  $\mu$ g of EndoFit™ Ovalbumin/Alhydrogel® adjuvant 2% (1:1, v/v) in a final volume of 100  $\mu$ l. Serum anti-OVA mIgG was monitored by ELISA (coated with ovalbumin at 10  $\mu$ g/ml in PBS). Alhydrogel® is a trademark, which belongs to Brenntag Biosector A/S and which is registered in a large number of countries and regions worldwide.

## TECHNICAL SUPPORT

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## RELATED PRODUCTS

Product	Description	Catalog Code
<b>Alum and emulsions</b>		
AddaVax™	Squalene-Oil-in-water	vac-adx-10
Adju-Phos® adjuvant	Aluminium phosphate gel	vac-phos-250
CFA	Complete Freund's adjuvant	vac-cfa-10
IFA	Incomplete Freund's adjuvant	vac-ifa-10
Quil-A® adjuvant	Saponin adjuvant	vac-quil
<b>PRR ligands</b>		
2'3'-cGAMP VacciGrade™	STING agonist	vac-nacga23
c-di-AMP VacciGrade™	STING agonist	vac-nacda
c-di-GMP VacciGrade™	STING agonist	vac-nacdg
Flagellin FliC VacciGrade™	TLR5 agonist	vac-fla
Gardiquimod VacciGrade™	TLR7 agonist	vac-gdq
Imiquimod VacciGrade™	TLR7 agonist	vac-imq
MPLA-SM VacciGrade™ (MPLA from <i>S.minnesota</i> )	TLR4 agonist	vac-mpla
N-glycolyl-MDP VacciGrade™	NOD2 agonist	vac-gmdp
ODN 1585 VacciGrade™	murine TLR9 agonist	vac-1585-1
ODN 1826 VacciGrade™	murine TLR9 agonist	vac-1826-1
ODN 2395 VacciGrade™	human/murine TLR9 agonist	vac-2395-1
ODN 2006 VacciGrade™	human TLR9 agonist	vac-2006-1
Pam3CSK4 VacciGrade™	TLR2 agonist	vac-pms
Poly(I:C) VacciGrade™	TLR3 agonist	vac-pic
R848 VacciGrade™	TLR7/8 agonist	vac-r848
TDB VacciGrade™	Mincle agonist	vac-tdb
<b>OVA antigens</b>		
EndoFit™ Ovalbumin	For <i>in vivo</i> use; endotoxin level <1EU/mg	vac-pova
Ovalbumin	For detection; Western, ELISA	vac-stova
Ova 257-264	For detection; ELISPOT	vac-sin
Ova 323-339	For detection; ELISPOT	vac-isq

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