



G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ [technical@GBiosciences.com](mailto:technical@GBiosciences.com)

A Geno Technology, Inc. (USA) brand name

# G-Alum™ Adjuvant Kit

(Cat. # 786-1216)



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## INTRODUCTION

Aluminum hydroxide preparation, commonly known as “Alum” is widely used adjuvant for increasing the immune response. Alum is generally less toxic and hazardous to animals, as well as researchers, than Freund’s adjuvants. Alum is an insoluble white colloidal suspension of aluminum hydroxide. When mixed with antigen, the antigen binds with the alum particles and becomes an insoluble antigen. The Alum-antigen suspension is injected into animals, where it forms a deposit of insoluble antigen at the injection sites. The alum bound insoluble antigen is localized for an extended period, hence, prolonging the interaction and uptake of antigens by immune responsive cells, such as T cells, B cells and antigen presenting cells (APCs). Alum stimulates pattern recognition receptors (PRRs) as well as a Th2 immune response.

Recent research has demonstrated that amongst the commercially available alums, such as the alums that are premade aluminum hydroxide gel preparations, are not as effective as alum-antigen precipitate - when antigen-aluminum hydroxide are coprecipitated gels<sup>1</sup>. Co-precipitation of antigen-alum induces formation of heterogeneous aluminum particles of 2-10µm, increasing the heterogeneity of antigen-alum; this heterogeneity enhances multiplicity of immune response.

Antigen adsorbs to the surface of aluminum adjuvants via hydrophobic and Vander Waals forces, via electrostatic attraction and by ligand exchange<sup>2</sup>. Aluminum hydroxide is positively charged at pH6-7.5 and attracts negatively charged protein.

G-Biosciences G-Alum™ Adjuvant Kit is alum-antigen precipitate kit where in alum-antigens complex is co-precipitated as a gel. Alum-antigen precipitates allow higher binding of antigens and consequently greater immune response including potent inducer of neutrophils, serum IL-5, IL-6, KC and MCP-1. G-Alum™ Adjuvant Kit does not contain any added inactive agents or preservatives that might undermine either antigen binding to alum or illicit adverse response to target antigens.

## ITEM(S) SUPPLIED

Description	Size
Aluminum Solution	100ml
Precipitating Agent	100ml

## STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival store the kit at room temperature. The kit is stable for 1 year if used as per instruction.

## ADDITIONAL ITEMS REQUIRED

- Desired antigen either coupled to carrier protein or used as it

## PROTOCOL

1. Prepare antigen in phosphate buffer saline and transfer appropriate volume of antigen preparation into a centrifuge tube.
2. Add 1 to 5 volumes of Aluminum Solution to the antigen and mix gently. For example, add 100-500  $\mu\text{l}$  Aluminum Solution for every 100  $\mu\text{l}$  antigen.  
**NOTE:** Do not use an antigen concentration of less than 100  $\mu\text{g}/\text{ml}$  Aluminum Solution.
3. Using an equivalent volume to the Aluminum Solution, add Precipitating Agent slowly and dropwise with mixing to the antigen aluminum solution. White antigen-alum precipitate will be formed.
4. Allow the antigen-alum precipitate to incubate at room temperature for 30 minutes with gently mixing.
5. Centrifuge the tube containing antigen-alum precipitate at 5000 x g for 5 minutes.
6. Remove and discard the supernatant.
7. Resuspend the antigen alum precipitate in appropriate volume of PBS with desired antigen concentration for injection.

**NOTE:** Appropriate volume of antigen-alum suspension and appropriate amount of antigen to be injected should be selected based on species to be injected. For example, for mice appropriate volume is 100-200  $\mu\text{l}$  and concentration of antigen is 50 to 100  $\mu\text{g}^4$ .

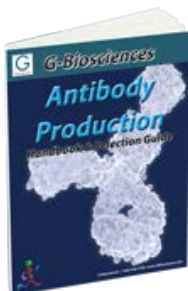
**NOTE:** Do not inject adjuvant intravenously as it can lead to anaphylaxis.

## REFERENCES

1. Derek, W. Cain., et al (2013). Vaccine 31 (4): 653-660.
2. Hem, S. L., and HogenEsch, H. (2007). Expert Rev. Vaccines 6, 685-698.
3. Erick B Lindblad (2004). Immunology and Cell biology 82: 497-505.
4. Harlow, E. and Lane, D. (1988). *Antibodies A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 56-100.

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