

Mouse Anti- Ovalbumin Monoclonal IgE Antibodies for Studying Allergic Diseases

Catalog # 3006-3008

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INTRODUCTION

Type I hypersensitivity, which is characterized by an allergic reaction immediately following contact with innocuous antigens, is a typical clinical feature of allergic diseases such as asthma, eczema, hay fever, or urticaria. This hypersensitivity mediated by the IgE antibodies is called “atopic reagin” and the clinical features of type I hypersensitivity are described as “atopy”. The high affinity receptor for IgE (FcεRI) expressed on mast cells and basophils is another critical component in allergic responses. IgE bound to FcεRI drastically up-regulates FcεRI expression on mast cells through stabilization and accumulation of FcεRI and contributes in further enhancing hypersensitivity responses to allergens (1). Specific allergens bound to IgE on cell surfaces will cross-link two FcεRIs and this linkage leads to the stimulation and degranulation of mast cells (2-3). This is associated with the release of a variety of proinflammatory mediators and cytokines such as histamine, proteolytic enzymes, heparin, and chemotactic factors, which cause the symptoms associated with type I hypersensitivity.

Mice are widely used experimental animals for studying pathogenesis and evaluating therapeutics in allergic disease models as a variety of inbred strains, transgenic mice, and even gene knockout mice are available (2-6). In order to induce allergic diseases in animals, immunize animals with an antigen adsorbed on aluminum hydroxide gel or expose animals to an aerosolized antigen repeatedly (7-9). In addition, IgE antibodies against specific antigens can sensitize animals by passive transfer *in vivo* as well as sensitize mast cells *in vitro* (8-10). For example, passive transfer of airway hyper-responsiveness by ovalbumin (OVA)-specific monoclonal IgE and IgG antibodies was reported by Oshiba *et al.*, although eosinophilic inflammatory response in the airway is modest despite high levels of serum IgE (8).

LIST OF IgE AND IgG MONOCLONAL ANTIBODIES AGAINST OVA

Chondrex, Inc. provides two IgE monoclonal antibodies specific to OVA, clone E-C1 and clone E-G6, in addition to one IgG1 monoclonal antibody against OVA, clone L71 (see Type I Hypersensitivity Research Products) (13-15). In general, the cross-linkage of IgE molecules bound to the receptor on mast cells by a polyvalent allergen is required to trigger degranulation of mast cells. Alternatively, IgG antibodies specific to the allergen can trigger the activation of mast cells by cross-linking two allergens bound by IgE antibodies on the mast cell surfaces (10). E-C1 alone is capable of inducing degranulation of mast cells *in vitro* (Figure 1), and severe hypersensitivity *in vivo* (Figure 2). Therefore, it is assumed that E-C1 might recognize repetitive epitopes of OVA. Furthermore, it has been reported that aggregated OVA, which carries multiple epitopes, increases the formation of cross-linked IgE on mast cell surfaces (16). On the other hand, E-G5 is not capable of inducing these hypersensitivity reactions by itself either *in vitro* or *in vivo* (Figures 1 and 2) and thus can be used as a control. Alternatively, Chondrex, Inc also provide a mouse IgE monoclonal antibody, isotype control, clone 2A101A12 as a negative control antibody.

The IgG1 monoclonal antibody, L71, can be used for a variety of research purposes such as studying the interference between allergen specific IgG and IgE antibodies as the pathogenic role of IgG in allergic diseases remains uncertain (11). For example, 1) IgG antibodies might compete with IgE antibodies and block the immediate hypersensitivity reactions, 2) IgG antibodies might stimulate IgE-mediated hypersensitivity reactions by cross-linking allergens bound to IgE on the surfaces of mast cells, or 3) IgG antibody-antigen immune complexes might stimulate IgE mediated hypersensitivity reactions by forming multivalent antigens which might bind to IgE on the surfaces of mast cells.

TYPE I HYPERSENSITIVITY RESEARCH PRODUCTS

Catalog #	Description	Antigen	<i>In Vitro</i> Mast Cell Activation	<i>In Vitro</i> Hypersensitivity Reaction	Quantity	Storage
3006	Mouse Monoclonal IgE Antibody E-C1	OVA & Aggregated OVA *	Yes	Yes	1 mg/vial Lyophilized with Mouse Serum Albumin	-20°C
3007	Mouse Monoclonal IgE Antibody E-G5	OVA Only **	No	No	1 mg/vial Lyophilized with Mouse Serum Albumin	-20°C
3008	Mouse Monoclonal IgG1 Antibody L71	OVA & Aggregated OVA	No	No	1 mg/vial Lyophilized with Mouse Serum Albumin	-20°C
7129	Mouse Monoclonal IgE Antibody 2A101A12 (Isotype Control)	Not Determined (Not OVA)	No	No	1 mg/ml in 0.05M PBS, 0.5 ml	-20°C

* Aggregated OVA can be prepared by mixing 2 ml of OVA solution (20 mg/ml in 0.05M PBS, pH 6.0) and 1 ml of 2.4% glutaraldehyde diluted with 0.05M PBS, pH 6.0 at room temperature overnight. Add 1 ml of 1M glycine to neutralize excess amounts of glutaraldehyde and react at room temperature for 30 minutes, then dialyze against 0.05M PBS, pH 7.2. In order to remove un-aggregated OVA, run Sephacryl S-400 gel filtration chromatography (15).

** Does not react with glutaraldehyde-aggregated OVA.

EXPERIMENTAL PROTOCOLS

A. Handling the IgE Antibody Solution

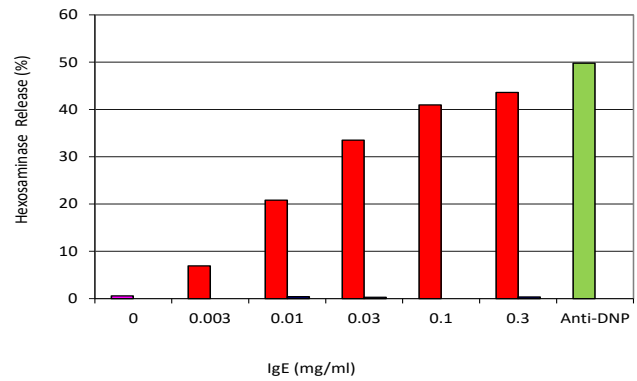
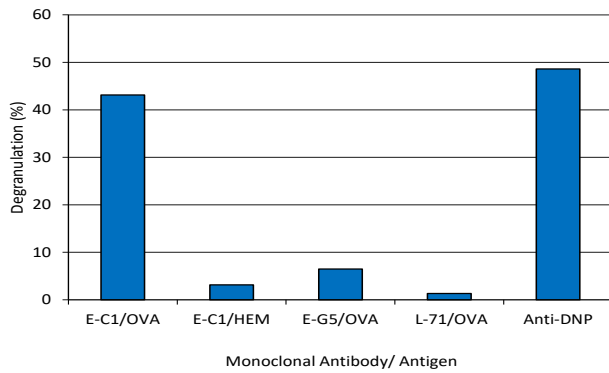
IgE antibody is biologically active at very low levels, so extra caution is required for handling and diluting IgE antibodies. In general, several 10 µg of protein will be adsorbed to 1 square cm of glass or plastic surfaces by non-specific hydrophobic binding. Therefore, if 1 ml of a 20 µg/ml IgE solution without blocking agents (such as PBS) is kept in a microcentrifuge tube, almost all the IgE antibodies will be adsorbed to the tube and no detectable IgE antibodies will remain in solution. Do not dilute IgE solutions to less than 100 µg/ml with PBS as it does not contain blocking agents. Add a suitable blocking agent to PBS, such as mouse serum albumin, bovine serum albumin (BSA), or other suitable proteins. IgE Dilution Buffer (Cat # 3009) is preferred for diluting IgE for both *in vitro* and *in vivo* experiments.

B. Assay for Degranulating Mast Cells *In Vitro*

- Culture mast cells such as rat basophilic leukemia cells (RBL-2H3) until the stationary phase. Re-suspend the cells at 2.5×10^5 cells/ml in Eagle's minimum essential medium containing 5% fetal calf serum (FCS) and transfer cells into 96-well (250 µl: 6.25×10^4 cell/well) or 24-well (1 ml: 2.5×10^5 cells/well) flat bottom cell culture plates, and continue to culture at 37°C overnight.
- Add IgE monoclonal antibodies diluted with PBS containing blocking agents or culture medium containing FCS to the wells (final concentration: 0-10 µg/ml of medium) and incubate for 1 hour.
- NOTE: Instead of adding IgE into culture media after overnight cell culture, IgE can be added to culture media at the start of cell culture. In this case, FcεRI expression could be significantly upregulated with the overnight culture. For example, it has been reported that FcεRI expression will be upregulated 2-fold in RBL cell lines and up to 32-fold on bone marrow-derived mast cells within 24-48 hours (1).

4. Wash IgE-sensitized cells 3 times with Tyrode's buffer (150 mmol/l NaCl, 2.5 mmol/l KCl, 12 mmol/l NaHCO₃, 2 mmol/l MgCl₂, 2 mmol/l CaCl₂, 1 mg/ml BSA, 1 mg/ml dextrose, pH 7.4).
5. Add 200 μ l (in 96-well plate) or 400 μ l (in 24-well plate) of OVA solution diluted with Tyrode's buffer (the final concentration of antigen: 0-5 μ g/ml) and culture for 1 hour.
6. Add 200 μ l (in 96-well plate) or 400 μ l (in 24-well plate) of a negative control antigen such as hen egg lysozyme (HEL) or BSA (5 μ g/ml) dissolved in Tyrode's buffer into the negative control wells to determine spontaneous release of β -hexosaminase.
7. Add 200 μ l (in 96-well plate) or 400 μ l (in 24-well plate) of 1% Triton X-100 solution dissolved in Tyrode's solution into 100% control wells to determine the total release of β -hexosaminase.
8. Assay β -hexosaminase activity in the culture supernatant using p-nitro-phenyl-N-acetyl b-D-glucosaminide as a substrate (11).

Figure 1 - Degranulation of Mast Cells by Monoclonal IgE Antibody



a) **Comparing the Three Monoclonal Antibodies:** RBL Cells cultured in a 24-well plate were sensitized by monoclonal antibody, E-C1 (1 μ g/ml), E-G5 (1 μ g/ml) and L71 (50 μ g/ml), respectively, and then activated by OVA (5 μ g/ml). As a positive control, a combination of anti-DNP IgE antibody (1:200 dilution in culture media) and DNP-HSA (0.05 μ g/ml) was used. E-C1 alone is capable of activating mast cells in antigen-specific manner, whereas E-G5 (IgE) and L-71 (IgG1) are not capable under the same conditions.

b) **Dose Response Effect of E-C1 and E-G5:** RBL Cells cultured in a 24-well plate were sensitized with E-C1 and E-G5 (0-0.3 μ g/ml), respectively and activated by OVA (5 μ g/ml). As a positive control, cells were sensitized by anti-DNP IgE antibody (1:200 dilution in culture media) and activated by DNP-HSA (0.05 μ g/ml).

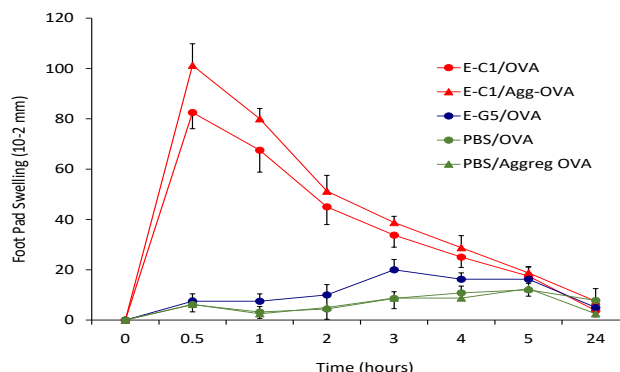
Note: Aggregated OVA causes an equal degree of degranulation of E-C1-sensitized mast cell at 1/5 concentration of OVA (data not shown).

C. Assay for Hypersensitivity Reactions *In Vivo*

Dilute IgE antibody solution (1 mg/ml) to 100 μ g/ml with PBS containing a blocking agent such as mouse serum albumin. Inject 100 μ l of the diluted IgE monoclonal antibody solution intravenously into mice (10 μ g/mouse). Inject 25 μ l of OVA (2 mg/ml in PBS) intradermally at the foot pad (50 μ g/foot pad) after 24 hours. Determine the swelling of the footpad by measuring the footpad thickness using a dial thickness gauge every 1-2 hours for 24 hours.

Figure 2 - Hypersensitivity Reaction in Foot Pad *In Vivo*

Balb/c mice intravenously received 10 µg of monoclonal antibody E-C1 or E-G5, then were challenged by OVA (50 µg) or aggregated OVA (Agg-OVA, 50 µg) by intradermal injection 24 hours later. Foot pad thickness was determined by a Loop Handle Dial Thickness Gauge and shown as mm.



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