Analyzing Antibodies in Intestinal Lavage

The gut barrier plays an important role in maintaining intestinal homeostasis by protecting the body from excess bacterial absorption. Immunoglobulins such as secretory IgA, IgG, and IgM aid in this protection. Chondrex, Inc. introduces a standard protocol for preparing intestinal lavage in mice, as well as ELISA kits to measure not only total immunoglobulin levels, but also antibody levels against antigens as anti-antigen antibody levels may indicate the state of intestinal immune function (1-3). For example, oral immunization of ovalbumin (OVA) with cholera toxin-adjuvant enhances intestinal IgA against OVA. This immune response can be evaluated by assaying for anti-OVA IgA antibody levels and comparing these to the total IgA levels. For more information about evaluating antibodies against specific antigens, bacteria, or toxins, please contact Chondrex, Inc. at support@chondrex.com.

Antigen-Specific Immunoglobulin ELISA Kits

Target	IgA	IgE	IgM	IgG	IgG1	IgG2a	IgG2b	IgG2c	IgG3
E. coli O111:B4	-	-	6209	6206	6207	6210	6211	-	6212
E. coli Lipopolysaccharide	-	-	-	6106	6107	6110	6111	-	-
Staphylococcal Enterotoxin A	-	-	-	6218	6219	6220	6221	-	-
Staphylococcal Enterotoxin B	-	-	-	6214	6215	6216	6217	-	-
Ovalbumin	3018	3010	3017	3011	3013	3015	3016	3029	-
House Dust Mite (HDM)	3046	3037	3036	3030	3034	3038	3035	-	3039
HDM Der p1	-	-	3049	3047	3048	-	-	-	3064
HDM Der p2	-	-	3068	3065	-	3066	3067	-	-
Gliadin	-	3050	3055	3051	3052	3053	3054	-	-
Crude Peanut Extract	3061	3063	3062	3056	3057	3058	3059	-	3060
Total Immunoglobulin	3019	3005	3024	3023	3025	3026	3027	-	3028

Intestinal Lavage Preparation Protocol in Mice

Mouse intestinal lavage for antibody analysis can be prepared through a method adapted from Lycke *et al.* (4).

1. Remove the entire mouse small intestine and clamp one end of the intestine (a scissor clamp can be used).
2. Carefully fill the intestine with 3 ml of a protease inhibitor solution (0.1 mg/ml trypsin inhibitor, 50 mM EDTA, and 1 mM PMSF in PBS) using a syringe with PTFE syringe tubing to avoid puncturing the intestine.
3. Clamp the other end of the intestine and incubate for 10 minutes at room temperature.
4. Transfer the intestinal contents to a test tube, vortex vigorously, and sonicate.
5. Centrifuge for 10 minutes at 1800 rpm at 4°C.
6. Transfer the supernatant to a new tube and mix with 10 µl of 5% sodium azide per 1 ml lavage.
7. Incubate for 15 minutes.
8. Add 50 µl of normal goat serum per 1 ml lavage. The mixture can be used for ELISA at a 1:1 dilution with each ELISA kit's respective Sample Dilution Buffer.

References

1. Tana, S. Watarai, E. Isogai, and K. Oguma. Induction of intestinal IgA and IgG antibodies preventing adhesion of verotoxin-producing Escherichia coli to Caco-2 cells by oral immunization with liposomes. *Lett Appl Microbiol* **36**, 135-139 (2003).
2. M. C. Thurnheer, A. Zuercher, J. Cebra, and N. Bos. B1 cells contribute to serum IgM, but not to intestinal IgA, production in gnotobiotic Ig allotype chimeric mice. *J Immunol* **170**, 4564-4571 (2003).
3. S. Senda, E. Cheng, and H Kawanishi. IgG in murine intestinal secretions. Aging effect and possible physiological role. *Scand J Immunol* **29**, 41-47 (1989).
4. N. Lycke, L. Erlandsson, L. Ekman, K. Schon, and T. Leanderson. Lack of J chain inhibits the transport of gut IgA and abrogates the development of intestinal antitoxic protection. *J Immunol* **163**, 913-919 (1999).