Analyzing Antibodies in Intestinal Lavage

Intestinal Immune System

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. Here, 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 intestinal antigens. Anti-antigen antibody levels may indicate the state of the 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. Please contact Chondrex, Inc. if you are interested in evaluating the antibodies against specific antigens, bacteria, or toxins.

Mouse ELISA Kits

Total Immunoglobulin ELISA Kits

Immunoglobulin Isotypes/Subtypes	IgA	IgE	IgM	IgG	IgG1	IgG2a	IgG2b	IgG3
Calalog #	3019	3005	3024	3023	3025	3026	3027	3028

Antigen-Specific Immunoglobulin ELISA Kits

Antigens		Immunoglobulin Isotypes/Subtypes							
		IgA	IgE	IgM	IgG	IgG1	IgG2a	IgG2b	IgG3
LPS	Calalog #	3019	3005	6028	6206	6107	6110	6111	6112
E. coli	Calalog #	Coming!	Coming!	6209	6106	6207	6210	6211	6212
OVA	Calalog #	3018	3005	3017	3011	3013	3015	3016	-
SEB	Catalog #	-	-	-	6214	6215	6216	6217	-
S. aureus	Calalog #	-	-	Coming!	6213	-	-	-	-

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.
4. Incubate for 10 minutes at room temperature.
5. Transfer the intestinal content to a test tube.
6. Vortex vigorously and sonicate.
7. Centrifuge for 10 minutes at 1800 rpm at 4°C.
8. Transfer the supernatant to a new tube and mix with 10 μl of sodium azide per 1 ml lavage.
9. Incubate for 15 minutes incubation.
10. Add 50 μl of goat serum per 1 ml lavage. The mixture can be used for ELISA with 1:1 dilution with Sample Dilution Buffer.

References

1. Tana, S et al. 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 et al. 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 et al. IgG in murine intestinal secretions. Aging effect and possible physiological role. Scand J Immunol 29, 41-47 (1989).
4. N. Lycke et al. Lack of J chain inhibits the transport of gut IgA and abrogates the development of intestinal antitoxic protection. J Immunol 163, 913-919 (1999).