

ImmuneTrack® Adaptive KIT

Adaptive Immunity Evaluation for salmonids

For the assessment of Diets, Supplements, Immunostimulants, Vaccines, Adjuvants and Others

- ✓ *Exclusive salmonid immune biomarker analysis*
- ✓ *Real Time PCR allows High sensitivity*
- ✓ *Immunity evaluation of different salmonid species*
- ✓ *Measures transcriptional changes of salmonid immune system*
- ✓ *A tool for design, evaluation and selection of diets, supplements, immunostimulant, vaccines, adjuvant and others*
- ✓ *High throughput assessment throughout the entire productive cycle*

ImmuneTrack®, a technological platform developed by ActivaQ which encompasses innate and adaptive immunity salmonid biomarkers for high throughput assessment throughout the entire productive cycle. Knowledge was acquired from several research projects, which were subsequently generated as services for the salmon industry and now can be purchased as a kit to use them in every lab.

We offer *ImmuneTrack® Innate KIT* and *ImmuneTrack® Adaptive KIT* which allow assessment of innate and adaptive immunity, respectively in salmonids. Immunity evaluation confers laboratory and industry solutions for the design, evaluation and selection of vaccines and diets amongst other treatments.

ImmuneTrack® Adaptive KIT by ActivaQ allows absolute quantification of the gene expression coding for the Cluster of Differentiation 4 (CD4), Interferon-gamma (INF- γ), Interleukin 10 (IL-10) and Transforming growth factor-beta (TGF- β) in salmonids, from tissue of spleen and kidney, through two step qRT-PCR using SYBR®Green and a calibration curve prepared from pure plasmidial DNA standards (pDNA).

To evaluate adaptive immunity biomarkers from CD4+ T cell populations, we also offer our exclusive *ImmuneTrack® Salmonid anti-CD4* (Purified Rabbit Polyclonal Antibodies) to perform the immunomagnetic separation of CD4+ cells

CD4

CD4 is a transmembrane glycoprotein, member of the immunoglobulin family, expressed on the cell surface of peripheral T cell and thymocytes. CD4 exerts its effect on T cell activation by two me-



chanisms, by binding to class II MHC molecules stabilizing the bond between T helper cells and antigen presenting cells (APC) and participates in signal transduction of T lymphocytes in response to antigen recognition.

IFN- γ

IFN- γ is a cytokine also called type II interferon (immunity). It is produced by Natural Killer cells (NK), mediating innate immunity. It is also produced by a subpopulation of CD4+ T cell and activated CD8+ T cells, thus participating in cell-mediated immunity. Its main effects are activating mononuclear phagocytes, increasing the expression of class I MHC molecules, increasing the gene expression involved in antigen presentation to stimulate the differentiation of T lymphocytes, and enhancing the cytolytic effect of NK cells and favour inflammatory reaction mediated by macrophages.

ImmuneTrack® Adaptive KIT

Adaptive Immunity Evaluation for salmonids

For the assessment of Diets, Supplements, Immunostimulants, Vaccines, Adjuvants and Others

IL-10

IL-10 is a cytokine produced by activated macrophages, some lymphocytes and non lymphocytic cells, such as keratinocytes. It inhibits the inflammatory response of innate immunity (affecting other cytokine production by macrophages, like TNF, chemokines, IL-1 and IL-2) and the response mediated by specific T cells (reducing the expression of MHC class II molecules and co-stimulating molecules, as B7-1 and B7-2).

IL-10 induces Th2 cell humoral immune response by generating B cell maturation and reduction of neutralizing antibodies

TGF- β

TGF- β is a cytokine produced by a T-cell subpopulation activated by antigens or activated mononuclear phagocytes. Its effects are pleiotropic, inhibiting the production of some cell types and stimulating others. Generally, TGF- β is considered an anti-cytokine that interrupts the immune and inflammatory response inhibiting T-cell proliferation by polyclonal mitogens, cytotoxic T lymphocytes maturation and macrophage activation. Also, it acts on polymorphonuclear lymphocytes and endothelial cell antagonizing the effects of proinflammatory cytokines.

Biomarkers and Immune Response

Antigen presentation and class II MHC immune response: The expression level of CD4 is an indicator of the activation of the class II MHC immune response.

T Helper 1 response: The expression level of INF- γ is an indicator of the Th1 response activation; responsible of cellular immunity.

T Helper 2 response: The expression level of this cytokine is an indicator of the Th2 response activation; responsible for humoral immunity.

Immunosuppressive response: IL-10 together with TGF- β or TGF- β alone expression, suggests an immunosuppressive response by blocking the activation of lymphocytes and monocyte derived phagocytes. The expression level of this cytokine is an indicator of the inhibitory effect of the immune response.

Moreover *ImmuneTrack® Adaptive KIT* allows determining the effect of treatments designed to stimulate adaptive immune response in salmonids, allowing an evaluation of a stimuli or a treatment, such as a vaccination.

Kit contents

- Plasmidial DNA (pDNA) standards, containing an insert of a salmonids conserved nucleotide region of each gene, allows absolute quantification of gene expression. The pDNA standards are associated to a solid matrix, that enable room temperature storage
- Specific primers to amplify each gene
- pDNA standards elution buffer
- RNA extraction recommendations and qRT-PCR thermal profile, with a range efficiency of 90-110%

Instructions

1. Extract total RNA from liver, spleen and kidney.
2. Perform an RT using random primers
3. Amplify (qPCR) genes using specific primers by SYBR®-Green, quantify the gene expression in the sample using pDNA calibration curve.