

ImmuneTrack® Innate KIT

Innate 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*
- ✓ *Earlier immunity stage evaluation*
- ✓ *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 evaluations confer laboratory and industry solutions for the design, evaluation and selection of vaccines and diets, amongst other treatments.

ImmuneTrack®Innate KIT quantifies by means of Real Time PCR (qRT-PCR) the expression of a set of genes (cytokines and other immune related factors) in salmonid tissue.



ImmuneTrack®Innate KIT by ActivaQ allows absolute quantification of the gene expression coding for the C3 complement protein, Interferon-alpha (INF- α), Interferon-induced myxovirus resistance protein (Mx) and Interferon-gamma (INF- γ) from spleen and kidney, through two step qRT-PCR using SYBR®Green and a calibration curve prepared from pure plasmidial DNA standards(pDNA).

C3

The C3 complement protein is a serum glycoprotein that is generated by proteolytic cleavage during complement activation in classical, alternative and lectine pathways. C3 participates in the innate and humoral responses, converging on the opsonization, phagocytosis promotion and the formation of membrane attack complex (MAC) that produces cellular lysis.

INF- α

Interferon alpha is one of the cytokines that mediate and regulate innate immunity, called type I interferons. Viral infection is the most important signal for production of interferon type I. The main functions of IFN- α are to inhibit viral replication, increase the lytic potential of Natural Killer cells (NK), modulate the expression of MHC class I molecules and inhibit cell proliferation.

MX

The MX protein belongs to the class of dynamin-like large guanosine triphosphatases (GTPases) known to be involved in intracellular vesicle trafficking and organelle homeostasis. MX has an important

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antiviral activity against a wide range of RNA virus. These viruses are inhibited at an early stage in their life cycle. Mx appears to detect viral infection by sensing nucleocapsid-like structures. As consequence, these viral components are trapped and sorted to locations where they become unavailable for viral genome replication and therefore prevent the generation of new virus particles.

IFN- γ

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

Biomarkers and Immune Response

Anti bacterial response: The expression level of C3 protein gene is an indicator of the ability of an individual to respond early to a microorganism infection, mainly bacteria.

Antiviral response: The expression level INF- α and Mx genes are an indicator of the ability of an individual to respond early to a viral infection.

Inflammatory response: The expression level of IFN- γ is an indicator of the ability of an individual to respond early to an intracellular infection through inflammatory reaction mediated by macrophages and NK cell activation that destroys infected cells.

Moreover *ImmuneTrack® Innate KIT* allows determining the effect of treatments designed to stimulate innate immune response in salmonids, such as diets, supplements, immunostimulant applications and vaccination.

Kit contents

- Plasmidial DNA standards (pDNA), 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 enables 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.