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**User's Manual**

# Pig MX1 ELISA Kit

REF **DEIASL207**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.


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# PRODUCT INFORMATION

## Intended Use

For the specific determination of porcine Mx1 in whole blood and other biological fluids.

## General Description

Porcine Mx1, or by its official name Interferon-induced GTP-binding protein Mx1, is also known by its alternative name Myxovirus resistance protein 1, UniProt accession number P27594.

The group of Mx proteins was originally discovered and identified in studies using mouse strains which are either resistant or susceptible to influenza (Horisberger 1983). This resistance is mediated by the type I interferon (IFNα/β) - induced Mx1 which has an intrinsic antiviral activity that is necessary and sufficient for protection against influenza virus. Mx1 is a dynamin-like GTPase with antiviral activity against a wide range of RNA viruses and some DNA viruses. Mx proteins have been found in all animal species tested so far.

Mx proteins are induced by type I IFN, by double-stranded RNA and some viruses in mammalian cells (Horisberger 1991). Expression can be rapid and abundant and remains localized intracellularly. The highest levels of Mx1 induction have been found in the target organs for virus replication (Horisberger 1989). With respect to this ELISA, this implies that the relevant cells – leukocytes - need to undergo lysis and maximum solubilization of their contents. This is achieved by adding a suitable lysis buffer to freshly drawn anticoagulated blood before analysis or frozen storage.

More than 30 different proteins are induced by IFNs. The measurement of interferon-induced proteins can be used to quantify the presence of biologically active interferons. Mx1 is particularly attractive in this regard since it can quickly increase to high levels of expression that may reach 1% of the total cytosolic protein (Horisberger 1992), and it is stable.

A rapid determination of porcine Mx1 should be useful in pig population screenings, as well diagnostically as prophylactically, for the treatment and handling of at-risk populations.

Immunohistochemical analysis of lung tissue after experimental infection of pigs with influenza virus showed distinct reactivity in epithelial cells and macrophages. Corresponding kinetics indicated maximal expression of Mx1 at days 1 and 2 post inoculation (Jung 2006).

### Functions

Mx proteins can be located in the cytoplasm or the nucleus, conferring protection against viruses that replicate in the respective compartment. Thus, human MxA is located in the cytoplasm while rodent Mx1 accumulates in the nucleus. The antiviral specificities reflect this local distribution to some degree, in that cytoplasmic Mx1 inhibits both cytoplasmically and nuclear replicating viruses while nuclear Mx1 does not affect viruses with an exclusively cytoplasmic replication cycle. The main target structure of Mx1 is the viral nucleoprotein (Dittmann 2008). However, Mx proteins interact with highly divergent target proteins underlying susceptibility to Mx1.

In humans and rodents, only MxA and Mx1, respectively, have detectable antiviral activity, while MxB and Mx2/Mx3 have none.

### Biochemistry

Mx proteins have a molecular weight of 70 to 80kDa. They are $Mg^{2+}$ - dependent GTPases (Horisberger

1992).

Under appropriate conditions, Mx1 forms highly ordered oligomers with ring-like shapes that may form tubular structures leading to the permeabilization or disintegration of the nucleocapsid.

A comparison of Mx sequences from mammals, birds and fish revealed a striking conservation of domains. They all maintain the tripartite ATP/GTP binding domain and the dynamin family signature in the amino terminal half of the protein. In the carboxyl terminal half of the Mx proteins are the localization signals and the leucine zipper motifs which account for the trimerization of Mx1 in the cell (Leong 1998).

**Pathological significance**

Just as the quantitation of acute phase proteins such as C-reactive protein or haptoglobin can indicate bacterial infection, inflammation or trauma, the measurement of a virally induced marker can indicate the health status of animals. Information on sub-clinical disease leading to poor growth rates on farms, or the identification of diseased animals at slaughter are relevant factors regarding animal welfare, profitability and consumer protection.

The expression of interferon-alpha (IFNα) and Mx1 mRNA was studied in formalin-fixed tissue sections of pigs experimentally infected with porcine reproductive and respiratory syndrome virus (PRRSV). There was good correlation in the expression of the two mRNAs in pathological lung samples and a strong indication that they reflect an early host defense reaction against the infection (Chung 2004).

Mx1 was also detected in Kupffer cells, lymphocytes and hepatocytes in the livers of pigs infected with hepatitis E virus (Lee 2009).

The antiviral activity of human MxA seems to be higher against influenza virus strains of avian than of human origin, indicating a possible property of MxA in limiting propagation of a particular virus in a foreign host (Dittmann 2008).

The interferon-inducible gene (IFI-78K gene) that codes for the human 78kDa homolog of mouse Mx1 is located on chromosome 21 together with the alpha/beta interferon (IFN-α/β) receptor. In human embryonic lung cells, the gene for human MxA is rapidly (minutes to hours) but transiently expressed in the continuous presence of IFNα. Translation initiates within a few hours of IFN exposure and tapers off within a day or so. However, the amount of MxA protein remains constant thereafter, suggesting that it has a high metabolic stability. Besides type I IFN, synthetic double-stranded RNA (dsRNA) is a good inducer of the IFI-78K gene, as well as influenza A (H7N1) virus and Newcastle disease virus (NDV), but not vesicular stomatitis virus (VSV) (Goetschy 1989).

In pigs, IFNα but not IFNγ induces protection against VSV and influenza A (H7N1), and both IFNs induced an antiviral state against mengovirus (Horisberger 1992b).

In cats, conjunctival cells and white blood cells express Mx1 upon exposure to recombinant feline interferon-omega through ocular or oral administration. The intensity of expression seems to depend on the dose of interferon-omega, site of administration, and cell type (Bracklein 2006).

## Principles of Testing

One-step non-competitive sandwich assay using a highly specific monoclonal antibody for antigen capture, and a second, peroxidasecoupled monoclonal antibody for detection. For use with anticoagulated lysed whole blood from pigs. Reagent limited with tetramethylbenzidine color reaction, including a stop reaction and reading at 450/630nm in a multititer plate reader.

## Reagents And Materials Provided

1. Ready-to-use precoated and stabilized microtiter plate and plate sealer (1 each per kit). Store at 4-8°C.
2. 1.3ml (each) of five different standard concentrations of recombinant porcine Mx1: 300, 75, 18.75, 4.69, and 1.17ng/ml. Ready for establishing a standard curve. Store frozen (-20°C).
3. 75µl of a peroxidase conjugated detection antibody (concentrated 100x). Spin briefly before opening to retrieve all the solution. Store at 4-8°C.
4. 50ml assay buffer (3x concentrated), a purple solution containing a blocking agent and a preservative. Store at 4-8°C.
5. 15ml lysis buffer, to be added to anticoagulated whole blood at a ratio of 1 in 20. Store at room temperature.
6. 1.5ml Tetramethylbenzidine (TMB) - $H_2O_2$ solution. Protect from light and store at 4-8°C.
7. Substrate Buffer, 30ml potassium citrate, pH 4.1. Store at 4-8°C.

   The kit is stable for 12 months after delivery when stored under the conditions indicated above.

## Materials Required But Not Supplied

STOP solution (1N sulfuric acid), plastic tubes for sample dilution; pipettes and microplate reader with 450nm filter.

## Storage

Store at 4-8°C.

## Plate Preparation

As an example, the following plate arrangement can be chosen, where St300 through St1.2 are the standards from 300ng/ml to 1.2ng/ml. Sa-1 through Sa-41 are samples in duplicates. Cont refers to a control sample with a known Mx1 content (not included in the kit).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | St300 | St300 | Sa-3 | Sa-3 | Sa-11 | Sa-11 | Sa-19 | Sa-19 | Sa-27 | Sa-27 | Sa-35 | Sa-35 |
| B | St75 | St75 | Sa-4 | Sa-4 | Sa-12 | Sa-12 | Sa-20 | Sa-20 | Sa-28 | Sa-28 | Sa-36 | Sa-36 |
| C | St18.8 | St18.8 | Sa-5 | Sa-5 | Sa-13 | Sa-13 | Sa-21 | Sa-21 | Sa-29 | Sa-29 | Sa-37 | Sa-37 |
| D | St4.7 | St4.7 | Sa-6 | Sa-6 | Sa-14 | Sa-14 | Sa-22 | Sa-22 | Sa-30 | Sa-30 | Sa-38 | Sa-38 |
| E | St1.2 | St1.2 | Sa-7 | Sa-7 | Sa-15 | Sa-15 | Sa-23 | Sa-23 | Sa-31 | Sa-31 | Sa-39 | Sa-39 |
| F | Blank | Blank | Sa-8 | Sa-8 | Sa-16 | Sa-16 | Sa-24 | Sa-24 | Sa-32 | Sa-32 | Sa-40 | Sa-40 |
| G | Sa-1 | Sa-1 | Sa-9 | Sa-9 | Sa-17 | Sa-17 | Sa-25 | Sa-25 | Sa-33 | Sa-33 | Sa-41 | Sa-41 |
| H | Sa-2 | Sa-2 | Sa-10 | Sa-10 | Sa-18 | Sa-18 | Sa-26 | Sa-26 | Sa-34 | Sa-34 | Cont | Cont |

## Reagent Preparation

Try to estimate how much of the various solutions you will need and prepare the actual volumes that will be required in one experiment. Duplicate testing of samples and standards is highly recommended. Allow the reagents to warm up to room temperature and prepare the following dilutions immediately before use:

**Assay Buffer:**

Dilute the concentrated purple stock solution with two parts purified water. Example: add 30ml water to 15ml stock solution to obtain 45ml assay buffer.

**Samples:**

Blood is collected into buffered EDTA (6-10mM final concentration) or Heparin (20-30 I.U. per ml final concentration) to prevent coagulation. 1 part lysis buffer is added to 20 parts whole anticoagulated blood, incubated for a few minutes, and stored frozen in aliquots. Example: Add 0.3ml lysis buffer to 6ml anticoagulated whole blood, mix gently and incubate for 3 minutes at room temperature, then aliquot and store at -20°C or lower. Use an aliquot only once and dilute it in appropriately diluted assay buffer. Recommended dilution is 1:20 for whole lysed blood.

**Standards:**

Standards should be stored at -20°C. They are ready for use after thawing and vortexing briefly. Return them to -20°C or lower after use.

**Detection reagent:**

Dilute reagent with assay buffer to give a 1:100 dilution. Example: add 52µl to 5.2ml assay buffer. 5.2ml are sufficient for one 96-well plate.

**Substrate dilution:**

To be prepared immediately before use. Bring substrate buffer to room temperature before use. The dilution is prepared by mixing 20 parts substrate buffer with 1 part TMB - H2O2 solution. Example: add 0.5ml TMB solution to 10ml buffer. Use within 15 minutes after preparation.

**STOP solution:**

Dilute sulfuric acid to a concentration of 1N. Example: Add 2.8ml 95-97% sulfuric acid to 100ml water (In this sequence. Do not add the water to the acid). 95-97% concentrated sulfuric acid (specific gravity 1.84) is 36N.

## Assay Procedure

1. a) Thaw samples, vortex them briefly and centrifuge for 5 minutes on a tabletop centrifuge at full speed. Dilute supernatant 1:20, e.g. add 40µl to 960µl assay buffer (working dilution).

   b) Thaw standards and vortex them briefly.
2. Add 50µl of each of the standard solutions to the corresponding wells in the microtiter plate, and 50µl assay buffer in the wells reserved for the blanks.
3. Add 50µl of appropriately diluted sample to the corresponding wells.
4. Add 50µl diluted detection reagent(1:100, see above) to each well. A stepper repeat pipette is ideally suited for this purpose. Seal the plate and incubate over night (15 - 17 hours) at 4-8 °C.
5. After incubation, wash wells 4 times (ideally with 0.1% Tween 20 in 0.9% NaCl, but isotonic saline or deionized water may be sufficient) and blot plate onto a soft absorbing paper to eliminate remaining water.
6. Immediately add 100µl substrate dilution (see above) to each well. Incubate for 10 minutes at room temperature. A blue color reaction occurs where porcine Mx1 is present.
7. Stop color reaction by adding 50µl stop solution to each well. Coloration turns from blue to yellow.
8. Read absorbance within one hour at 450nm with reference wavelength set to 630-650nm.

## Calculation

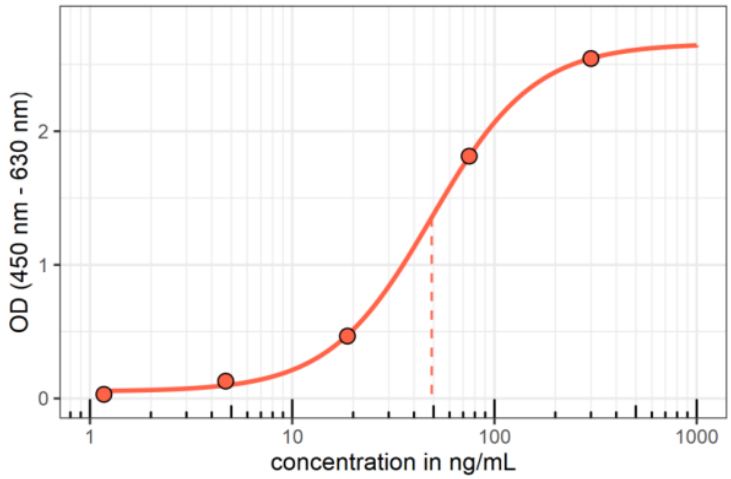
Means are formed from duplicates and the content in the samples is calculated from the standard curve with the help of a microplate calculation software (e.g. Softmax, Molecular Device) or manually. Sample dilutions which lie outside of the standard range should be repeated with the appropriate dilution.

## Interpretation Of Results

Normal values of blood from apparently healthy pigs (slaughterhouse) typically give values below 2.0µg/ml. Animals tested positively for PRRSV typically give values above 3.5µg/ml.

## Typical Standard Curve

Typical standard curve for the range 1.2 - 300ng/ml:




## Limitations

The tap water used for washing the plates may contain high chlorine levels which negatively affect the performance.

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