

# ELISA KIT FOR HUMAN H-FICOLIN



**Catalog nr** HK340 (2 x 96 determinations)

**Description** Ficolins are a group of proteins containing both a collagen-like domain and a fibrinogen-like domain. Three forms of Ficolin have been identified in humans: L-Ficolin, M-Ficolin and H-Ficolin. H-Ficolin, also known as Hakata-antigen or Ficolin-3, is composed by a collagen-like strand and three C-terminal recognition domains which bind to acetyl groups on microbial surfaces such as GlcNAc or GalNAc. H-Ficolin is synthesized both in the liver, from where it is secreted into the blood circulation, and in the lung. Similar to MBL and L-Ficolin, H-ficolin relies on MBL-associated serine protease 2 (MASP-2) for activation of the complement system. After binding of H-ficolin/MASP-2 complexes to microbial surfaces, MASP-2 sequentially cleaves C4 and C2, thereby generating the C3 convertase C4bC2b, which finally leads to opsonization and direct lysis of pathogens and recruitment of inflammatory cells.

H-ficolin is present in serum at mean concentration of 15 µg/ml, with only minor variations. H-ficolin was present in all sera from more than 150,000 individuals tested, except in some systemic lupus erythromatosis patients. Approximately 5% of systemic lupus erythromatosis patients were found to be H-ficolin negative, probably owing to the presence of anti-H-ficolin autoantibody. In 398 patients with other autoimmune diseases, H-ficolin was always present. In liver disease the serum levels decreased with increasing severity of cirrhosis.

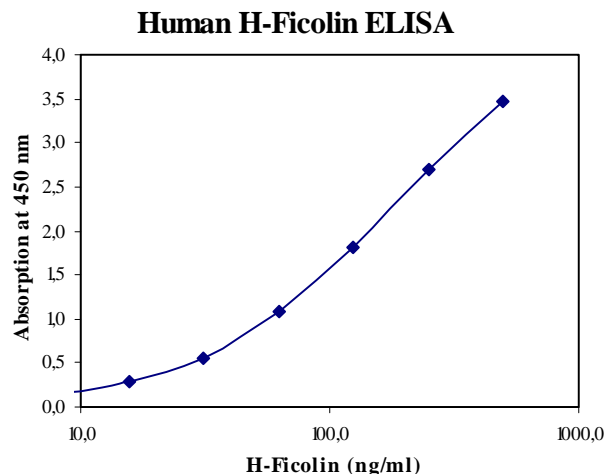
H-ficolin bound to a population of late apoptotic cells, while a strong and uniform binding to necrotic cells was observed. The binding properties differed from those of MBL and L-ficolin. H-ficolin binding to late apoptotic cells resulted in a significant increase in adhesion/uptake by macrophages.

**Aliases** Hakata-antigen, Ficolin-3

**Application** The human H-Ficolin ELISA has been developed for the quantitative measurement of natural H-Ficolin in plasma, and serum. In plasma samples, H-Ficolin can be measured accurately if samples are diluted at least 150 times. Most reliable results are obtained if EDTA plasma is used.

- Features**
- Minimum concentration which can be measured is 3 ng/ml human H-Ficolin
  - Measurable concentration range of 3-500 ng/ml.
  - Working volume of 100 µl/well.

**Typical standard curve**



- Principle**
- The human H-Ficolin ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 3½ hours.
  - The efficient format of 2 plates with twelve disposable 8-well strips allows free choice of batch size for the assay.
  - Samples and standards are captured by a solid bound specific antibody.
  - Biotinylated tracer antibody will bind to captured H-Ficolin.
  - Streptavidin-peroxidase conjugate will bind to the biotinylated tracer antibody.
  - Streptavidin-peroxidase conjugate will react with the substrate, tetramethylbenzidine (TMB).
  - The enzyme reaction is stopped by the addition of citric acid.
  - The absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the

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absorbance (linear) versus the corresponding concentrations of the H-Ficolin standards (log).

- The human H-Ficolin concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

### Storage and stability

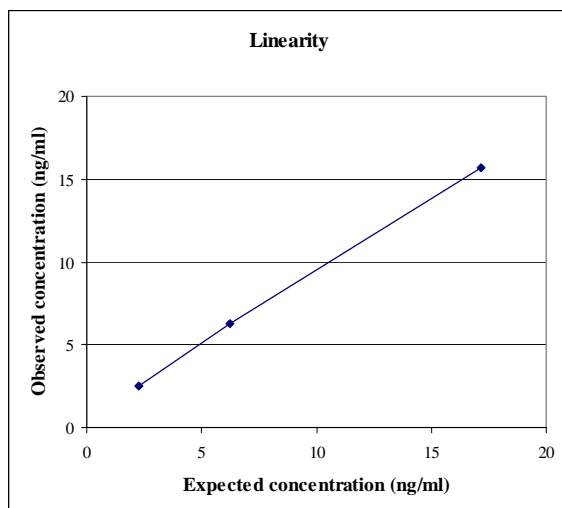
Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least six months. After reconstitution the reagents are stable for 1 month if stored at 2-8°C, except for the standard. After reconstitution, the standard must be used within 15 minutes. For longer stability, we recommend to store aliquots at -20°C.

### Recovery

Normal human blood samples (plasma), containing baseline levels of 500 ng/ml were spiked with recombinant H-Ficolin in concentrations of 6.5 and 100 ng/ml. Samples with and without H-Ficolin were incubated for 1 hour at room temperature. Samples were measured using the ELISA. Values for H-Ficolin ranged between 94 % and 115%.

### Linearity

The linearity of the assay was determined by serially diluting a sample containing 98.1 ng/ml human H-Ficolin. The diluted samples were measured in the assay. The line obtained a slope of 0.883 and a correlation coefficient of 1.0.



### Precautions

For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and Federal rules in the use of this product. Hbt is not responsible for any patent infringements that might result with the use of or derivation of this product.

### References

1. Runza, V et al; Ficolins: novel pattern recognition molecules of the innate immune response. *Immunobiol* 2008, 213: 297
2. Honore, C et al; The innate immune component Ficolin 3 (Hakata Antigen) mediates the clearance of late apoptotic cells. *Arthritis & Rheumatism* 2007, 56: 1598
3. Thiel, S; Complement activating soluble pattern recognition molecules with collagen-like regions, mannan-binding lectin, ficolins and associated proteins. *Mol Immunol* 2007, 44: 3875
4. Kuraya, M et al; Specific binding of L-ficolin and H-ficolin to apoptotic cells leadsto complement activation. *Immunobiol* 2005, 209: 689
5. Holmskov, U et al; Collectins and Ficolins: Humoral Lectins ofthe Innate Immune Defense. *Annu Rev Immunol* 2003, 21: 547

### Also available

HK322	Human MBL ELISA kit, 2 x 96 determinations
HK326	Human MASP-2, ELISA kit, 2 x 96 determinations
HK327	Human functional MBL/MASP-2, ELISA kit, 2 x 96 determinations
HK328	Human TCC, ELISA kit, 2 x 96 determinations
HK336	Human L-Ficolin ELISA kit, 2 x 96 determinations