BioLab Assays

Human Insulin-like Growth Factor-binding Protein 7 (IGFBP7) ELISA

Cat. No.: BA1024

Enzyme Immunoassay for the quantitative determination Insulin-like Growth Factor-binding Protein 7 in human serum, plasma, and urine.

IGFBP7 is a member of the insulin superfamily of growth promoting peptides, and is one of the most abundant and common growth factor polypeptides. IGFBPs are a class of secreted proteins that is able to interact with many ligands other than IGFs.[1]

Currently, IGFBP-7 has been used as an early diagnosis and prognostic marker for acute renal insufficiency (AKI). IGFBP-7, and TIMP-2 concentrations in the urine of children after receiving injections of contrast medium increased faster than SCr. The combination of IGFBP-7 and TIMP-2 was better than either analyte alone (the specificity was 80.0%, and the sensitivity was 81.2%).[2] Based on the results of two studies, the urine compound of TIMP-2 and IGFBP-7 became US Food and Drug Administration (FDA)-approved biomarker for risk assessment of AKI in ICU patients.[1]
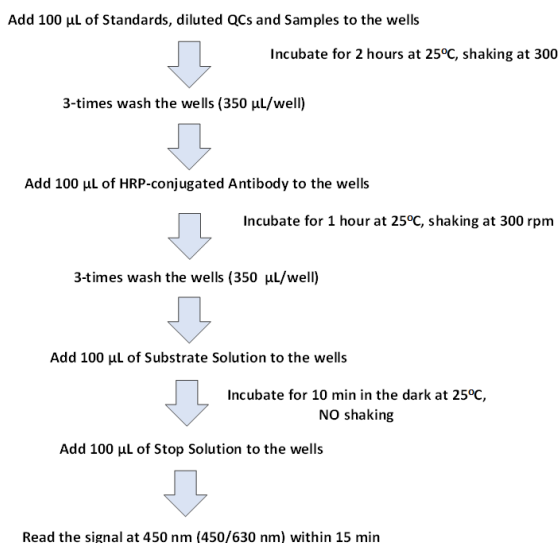
IGFBP-7 has also been proposed as a potential prognostic biomarker in heart failure (HF). It provided prognostic information incremental to clinical variables, NT-proBNP, and hsTnT.[3]

PRINCIPLE OF INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN 7 ELISA

The microtiter plate is coated with the antibody specifically binding Insulin-like Growth Factor-binding Protein 7. The human serum, plasma or urine is incubated in the plate with the capture antibody.

The specimen is washed out and the specifically bound protein is incubated with HRP-labelled detection antibody. Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).

The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of the Insulin-like Growth Factor-binding Protein 7 the specimen. The concentration of Insulin-like Growth Factor-binding Protein 7 in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.

Add 100 µL of Standards, diluted QCs and Samples to the wells

⬇ Incubate for 2 hours at 25ºC, shaking at 300

3-times wash the wells (350 µL/well)

⬇

Add 100 µL of HRP-conjugated Antibody to the wells

⬇ Incubate for 1 hour at 25ºC, shaking at 300 rpm

3-times wash the wells (350 µL/well)

⬇

Add 100 µL of Substrate Solution to the wells

⬇ Incubate for 10 min in the dark at 25ºC, NO shaking

Add 100 µL of Stop Solution to the wells

⬇

Read the signal at 450 nm (450/630 nm) within 15 min

Kit Contents

Item	Qty.
Antibody Coated Microtiter Plate	96 wells
Antibody-HRP Conjugate	13 mL
Master Standard (lyophilized)	1 vial
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer	2x13 mL
Wash Buffer 15x conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

MATERIAL REQUIRED BUT NOT SUPPLIED

1. Glassware and test tubes
2. Microtiter plate washer
3. Precision pipettes (various volumes) with tips
4. Orbital shaker
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

WARNINGS AND PRECAUTIONS

1. For research use only
2. For professional laboratory use
3. The reagents with different lot numbers should not be mixed
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25ºC ± 2ºC).

STORAGE CONDITIONS

1. The kit must be stored at 2 – 8ºC.
2. The opened components can be stored for one week at 2 – 8ºC.

PREPARATION OF REAGENTS

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25ºC ± 2ºC.

Preparation of Standards

Reconstitute lyophilized Human IGFBP7 Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min prior to use. The concentration of human IGFBP7 in Master Standard is 4 ng/mL.

Prepare set of Standard solution as follows:

Use the Master Standard for serial dilution (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 4 ng/mL (lyophilized)	See CofA	4 ng/mL
Std2	250 µL of Std1	250 µL	2 ng/mL
Std3	250 µL of Std2	250 µL	1 ng/mL
Std4	250 µL of Std3	250 µL	0.5 ng/mL
Std5	250 µL of Std4	250 µL	0.25 ng/mL
Std6	250 µL of Std5	250 µL	0.125 ng/mL
Blank	-	200 µL	0 ng/mL

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Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls with deionized/distilled water, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min and dilute them 1:80 in Dilution Buffer, prior to use, see Preparation of samples.

Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15x conc. to 700 mL of deionized/ distilled water (dH$_2$O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

Preparation of samples

Human serum, plasma or urine may be used with this assay. For long-term storage the samples should be frozen at minimum -70°C. Lipemic or haemolytic samples may cause false results.

Recommended dilution for serum, plasma and urine 1:80. It is recommended to use the two-step dilution.
Dilution A (8x) for both singlets and duplicates: 10 µL of samples + 70 µL of Dilution Buffer.
Dilution B (10x): 20 µL of Dilution A + 180 µL of Dilution Buffer, for singlets; 30 µL of Dilution A + 270 µL of Dilution Buffer, for duplicates.
Do not store the diluted samples.

ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for 2 **hours** at 25°C ±2°C, shaking at 300 rpm.
3. Wash the wells 3-times with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of HRP-labelled Antibody Conjugate into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µL Substrate solution, incubate for **10 min,** at 25°C ±2°C. Avoid exposure to the light during this step.
7. Pipette 100 µL of STOP solution.
8. Read the signal at 450 or 450/630 nm within 10 min.

PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:80 as recommended and assayed. The results are multiplied by the dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human IGFBP7 giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 0.031 ng/mL of sample.

2. Precision

Intra-assay

Sample	Mean (ng/mL)	SD	CV (%)
1	84.2	4.2	5
2	90.0	4.3	5

Inter-assay (Run – to – run)

Sample	Mean (ng/mL)	SD	CV (%)
1	77.6	3.7	5
2	80.4	8.3	10

3. Accuracy

Dilution linearity

Sample	Dilution	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1		85.8	-	-
	2x	41.0	42.9	96
	4x	17.7	21.4	83
	8x	8.56	10.7	80
2		93.4	-	-
	2x	43.5	46.7	93
	4x	20.0	23.3	86
	8x	9.4	11.7	80

Spiking Recovery

Sample	Spike (ng/ml)	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1	-	68.8	-	-
	160	250.2	228.8	109
	80	162.4	148.8	109
	40	121.9	108.8	112

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.

Human IGFBP 7 Standard Curve
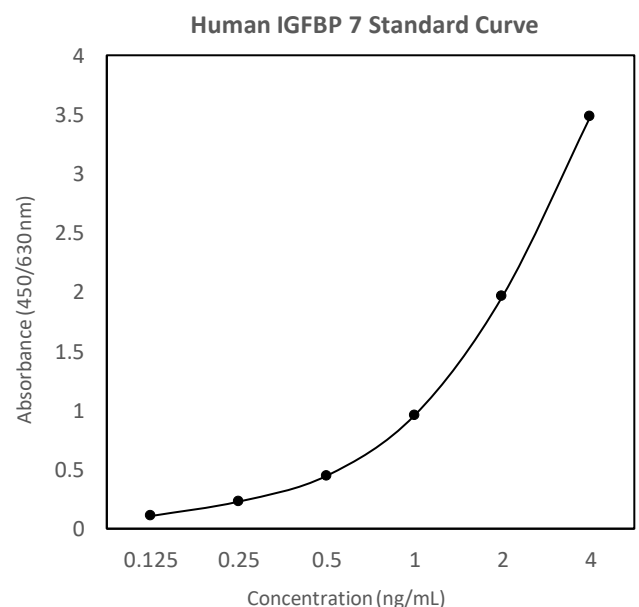
RESOURCES

[1]Wang S, Chi K, Wu D, Hong Q. Insulin-Like Growth Factor Binding Proteins in Kidney Disease. Front Pharmacol. 2021 Dec 22;12:807119. doi: 10.3389/fphar.2021.807119. PMID: 35002740; PMCID: PMC8728008.

[2]Sun Q, Kang Z, Li Z, Xun M. Urinary NGAL, IGFBP-7, and TIMP-2: novel biomarkers to predict contrast medium-induced acute kidney injury in children. Ren Fail. 2022 Dec;44(1):1201-1206. doi: 10.1080/0886022X.2022.2075277. PMID: 36120960; PMCID: PMC9518296.

[3]Adamson C, Welsh P, Docherty KF, de Boer RA, Diez M, Drożdż J, Dukát A, Inzucchi SE, Køber L, Kosiborod MN, Ljungman CEA, Martinez FA, Ponikowski P, Sabatine MS, Morrow DA, Lindholm D, Hammarstedt A, Boulton DW, Greasley PJ, Langkilde AM, Solomon SD, Sattar N, McMurray JJV, Jhund PS. IGFBP-7 and Outcomes in Heart Failure With Reduced Ejection Fraction: Findings From DAPA-HF. JACC Heart Fail. 2022 Oct 20:S2213-1779(22)00571-6. doi: 10.1016/j.jchf.2022.09.004. Epub ahead of print. PMID: 36592046.