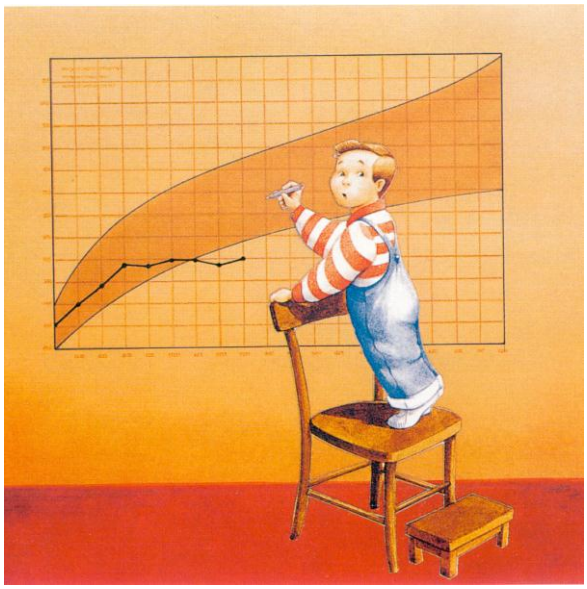
Acid-labile Subunit ELISA E35



IGF-I, IGFBP-3 and the acid labile subunit (ALS) build up the so called ternary complex, the major storage of IGF-I in circulation. ALS expression is located in the liver and controlled by human growth hormone. Thus, determination of serum ALS levels could be helpful in the differential diagnosis of patients with idiopathic IGF-I deficiency.

This test system is based on polyclonal antibodies, highly specific for the acid labile subunit. Peptides of previously published sequences have been used for immunization of rabbits[1]:

N-terminal:
ADPGTPGEAEGPACPAACVCSYD DDADELSVFCS
C-terminal:
YNNITCASPPEVVGLDLRDLSEAHFAPC

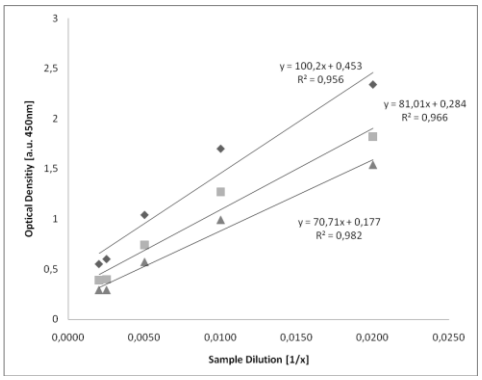
Specimen

Human serum and plasma samples can be used in this assay. No influence of Heparin (30 IE/mL), EDTA (6.8 mM) and NaCitrate (0.015 M) on the measurement of ALS has been detected by recovery experiments.

Interference	% of control
Triglycerides [100 mg/mL]	100
Hemoglobin [1 mg/mL]	105
Bilirubin [200 µg/mL]	94

Indication

ALS is a marker protein for growth hormone action. It could therefore be used for therapy surveillance and initial diagnostics of growth hormone deficiency or acromegaly.



Linearity, measured signal intensity [OD_{450}] of differentially diluted samples. The recommended dilution is 1:150 (0.007).

Sample Stability

Storage at 25°C up to 3 days
Storage at -20°C up to 2 years
Not more than 5 freeze/thaw cycles!

Assay Featuters

- ✓ Calibration against recombinant ALS
- ✓ native human ALS standard: 0 – 200 ng/mL
- ✓ Assay Range 0.53 – 30 000 ng/mL
- ✓ analytical sensitivity of 0.53 ng/mL
- ✓ high precision:
  - inter-assay variance ≤ 8.0%
  - intra-assay variance ≤ 6.8%
- ✓ excellent linearity
- ✓ 2 control sera for GLP conformity
- ✓ Fast: Incubation Time of 3 hours

Literature

(1) Stadler S, Wu Z, Dressendörfer RA, Morrison KM, Khare A, Lee PDK, **Strasburger, CJ**: J Immunological Methods 252 (2001) 73-82
(2) Morrison KM, **Bidlingmaier M**, Stadler S, Wu Z, Skriver L, **Strasburger CJ**: European Journal of Endocrinology (2007) 156

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Mediagnost Acid-labile Subunit ELISA, E35

Reconstitution/ Dilution of Reagents		
Standards A-F	Reconstitution in **Sample Buffer PP** (red)	**1000 µLeach**
Control Serum KS1/KS2	Reconstitution in **Sample Buffer PP** (red)	**250 µL each**
Antibody Conjugate AK	dilute before use: **1:50** in **Dilution Buffer VP**	**1:50**
Washing Buffer WP	Dilute in **A. dest.** (e.g. add the complete contents of the flask 50 mL into a graduated flask and fill with A.dest. to 1000 mL)	**1:20**
Sample and Control Sera KS1 &KS2 Dilution: 1:150 in Sample Buffer PP (red colored; e.g. **10** µL in **1490 µl PP**). **mix directly and use within max. 60 min.** Use **50 µL per determination** (pipetting control= red coloration)		
Before assay procedure bring all **reagents** to **room temperature**		

Pipette	Reagents	Well Positions
50 µL	**1:50 diluted Antibody Conjugate (AK)**	Pipette **in all required number of wells**
50 µL	Standard **A** (**0 ng/mL**)	A1 and A2
50 µL	Standard **B** (**7.5 ng/mL**)	B1 and B2
50 µL	Standard **C** (**31.25 ng/mL**)	C1 and C2
50 µL	Standard **D** (**62.5 ng/mL**)	D1 and D2
50 µL	Standard **E (125 ng/mL)**	E1 and E2
50 µL	Standard **F** (**200 ng/mL**)	F1 and F2
50 µL	Diluted Control Serum **KS1**	G1 and G2
50 µL	Diluted Control Serum **KS2**	H1 and H2
50 µL	Diluted Sample	Pipette sample in the rest of the wells according to requirements
Cover the wells with the sealing tape		

Incubation: 2 h at RT**,** 350 rpm

5x 300 µL	Aspirate the contents of the wells and wash **5x** with **300 µL** each **WP/well**	each well
100 µL	**Enzyme Conjugate EK**	each well

Incubation: 0.5 h at RT, 350 rpm

5x 300 µL	Aspirate the contents of the wells and wash **5x** with **300 µl** each **WP/well**	each well
100 µL	Substrate Solution **S**	each well

Incubation: 30 min **in** the dark **at RT**

100 µL	Stop Solution **SL**	each well
Measure the absorbance within 30 min at **450 nm** (≥590 nm Reference)		

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