Anti-ATP4B ELISA (IgG)

- **Reliable detection and quantification of autoantibodies against parietal cells**
- **Supports the diagnosis of autoimmune gastritis or pernicious anaemia**
- **Fully automatable**

Technical data

Antigen	Recombinantly produced β-subunit of H^+/K^+-ATPase (ATP4B)
Calibration	Quantitative, in relative units per millilitre (RU/ml)
Result interpretation	EUROIMMUN recommends interpreting results as follows:
	< 20 RU/ml: negative
	≥ 20 RU/ml: positive
	Recommended upper threshold of the normal range (cut-off value): 20 RU/ml
Sample dilution	Serum or plasma, 1 : 101 in sample buffer
Reagents	Ready for use, with the exception of the wash buffer (10 x)
Test procedure	30 min / 30 min / 15 min (room temperature)
Measurement	450 nm, reference wavelength between 620 nm and 650 nm
Test kit format	96 single break-off wells; kit includes all necessary reagents
Order number	**EA 1361-9601-1G**

Clinical significance

Autoimmune gastritis (AIG) is a chronic inflammation of the stomach mucous membrane, which may lead to atrophic gastritis with malabsorption, affecting the uptake of iron and vitamin B12. Young patients develop iron deficiency anaemia. Due to the vitamin B12 deficiency, pernicious anaemia (PA) develops over many years.

In most patients AIG proceeds asymptomatically over many years to the late stage of atrophy. Symptoms of PA are anaemia, fatigue, drowsiness and tachycardia. Vitamin B12 deficiency inhibits the DNA synthesis, resulting in the formation of megaloblasts, e.g. in the bone marrow and gastrointestinal epithelium. This leads to malabsorption and diarrhoea with weight loss, anorexia, glossitis, icterus and neurological abnormalities.

Autoantibodies against parietal cells (APCA) are the most sensitive biomarkers for AIG. They occur in 80 to 90% of PA patients, especially in the early stage of the disease, and can be found several years before the onset of clinical symptoms. With progressing gastritis and the loss of parietal cells, APCA are less frequently found.

APCA can also occur in other autoimmune diseases, e.g. in Hashimoto's thyroiditis, vitiligo, type 1 diabetes mellitus, Sjögren's syndrome, coeliac disease and Addison's disease. They are also found in around 20% of patients with *Helicobacter pylori* infections. APCA rarely occur in the healthy population, but their frequency increases with the patients' age.

The diagnosis of PA is based on the detection of megaloblastic anaemia, a low vitamin B12 level in the serum, stomach mucosal atrophy and antibodies against parietal cells and intrinsic factor. The diagnostic sensitivity of APCA for PA is approx. 90%. The detection of APCA against the extracellular domain of the β-subunit of H^+/K^+-ATPase (ATP4B) allows a particularly specific diagnosis. By using the Anti-ATP4B ELISA from EUROIMMUN the specificity can be significantly increased, with any loss in the specificity, compared to the Anti-PCA ELISA. Asymptomatic AIG is diagnosed through the detection of APCA. Antibodies against intrinsic factor indicate developing or established PA.

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Reference range

Levels of anti-ATP4B antibodies were determined in 200 sera from healthy blood donors of between 18 and 69 years of age (55 women, 145 men) using the EUROIMMUN ELISA. With a cut-off value of 20 RU/ml, 1% of the blood donors were anti-ATP4B positive (IgG). No differences with respect to age or gender were observed.

Blood donors (n = 200)			
Percentile (%)	98	99	100
Cut-off (RU/ml)	14.97	19.28	169.23

Precision

Studies on the intra-laboratory and inter-lot precision were performed according to the CLSI guideline EP05-A3. Six samples (reactivity distributed over the entire measurement range) were measured. The precision is given as standard deviation (SD) and coefficient of variation (CV).

Intra-lab precision

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
Mean value (RU/ml)	6.49		18.36		14.85		22.16		97.48		157.55	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Repeatability	0.202	3.1	0.708	3.9	0.442	3.0	1.535	6.9	3.344	3.4	3.834	2.4
Between-run	0.452	7.0	0.737	4.0	0.499	3.4	1.322	6.0	2.231	2.3	4.429	2.8
Within-day	0.495	7.6	1.022	5.6	0.667	4.5	2.026	9.1	4.020	4.1	5.858	3.7
Between-day	0.131	2.0	0.0	< 0.1	0.322	2.2	0.0	< 0.1	2.098	2.2	8.585	5.4
Within-lab	0.513	7.9	1.022	5.6	0.740	5.0	2.026	9.1	4.534	4.7	10.393	6.6

Inter-lot precision

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
Mean value (RU/ml)	6.75		19.21		15.31		22.92		97.43		157.28	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Repeatability	0.182	2.7	1.018	5.3	0.783	5.1	1.710	7.5	4.603	4.7	5.628	3.6
Between-run	0.321	4.8	0.956	5.0	0.558	3.6	1.789	7.8	4.007	4.1	6.597	4.2
Within-day	0.226	3.4	0.0	< 0.1	0.0	< 0.1	0.0	< 0.1	0.0	< 0.1	0.0	< 0.1
Within-lot	0.433	6.4	1.396	7.3	0.961	6.3	2.475	10.8	6.102	6.3	8.671	5.5
Between-lot	0.0	< 0.1	0.014	0.1	0.0	< 0.1	0.000	< 0.1	4.025	4.1	4.296	2.7
Reproducibility	0.433	6.4	1.396	7.3	0.961	6.3	2.475	10.8	7.310	7.5	9.677	6.2

Diagnostic specificity and sensitivity

Samples from 160 patients with different autoimmune diseases were investigated using the EUROIMMUN Anti-ATP4B ELISA (IgG). The specificity of the ELISA was 95.0% (see table).

Samples from 29 patients with autoimmune gastritis were analysed using the EUROIMMUN Anti-ATP4B ELISA (IgG). The sensitivity amounted to 96.6%.

Cohort	Total (n)	Positive in Anti-ATP4B ELISA (IgG)	
		n	%
Crohn's disease	30	1	3.3
Ulcerative colitis	30	1	3.3
Type 1 diabetes mellitus	30	3	10.0
Autoimmune thyroiditis	20	1	5.0
Coeliac disease	30	0	0
Sjögren's syndrome	20	2	10.0
Specificity	160	8	95.0

Method comparison

72 samples characterised as positive with the EUROIMMUN Anti-PCA ELISA (IgG) were investigated using the EUROIMMUN Anti-ATP4B ELISA (IgG). There was an agreement of 94% between the Anti-ATP4B ELISA (IgG) and the Anti-PCA ELISA (IgG) (reference).

n = 72		EUROIMMUN Anti-PCA ELISA (IgG)	
		positive	negative
EUROIMMUN Anti-ATP4B ELISA (IgG)	positive	68	0
	negative	4*	0

* None of the samples was positive in the EUROIMMUN Anti-Intrinsic Factor ELISA (IgG). One sample was weakly positive in the EUROIMMUN IIFT: Stomach (Monkey) (IgG) (titer 1:10).

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