# **Tryptophan in stool**



## Neurobalance in the gut

## **IDK®** Tryptophan ELISA

# Competitive ELISA for the quantitative determination of L-tryptophan in stool

- Tryptophan deficiency begins in the gut
- The healthy microbiome regulates the intestinal tryptophan metabolism
- In IBD, tryptophan levels are found to be low

Also available: Tryptophan ELISA for the determnation in serum, plasma and dried blood samples (K 7730)





### Low levels of tryptophan in inflammatory bowel diseases (IBD)

The essential amino acid L-tryptophan stems mainly from the diet and is resorbed in the small and large intestines via amino acid transporters. Tryptophan is then degraded by different enzymes to several important metabolites, mainly kynurenine, kynurenic acid, quinolinic acid, NAD (nicotinamide adenine dinucleotide) and small amounts of serotonin (*Fig.* 1).

Degradation of trytophan mainly occurs along the kynurenine pathway and comprises at least 90% of tryptophan catabolism. The metabolisation of tryptophan to kynurenine by the enzyme indoleamine 2,3-dioxygenase 1 (IDO1) is the initial and rate-limiting step. Modulation of IDO1 regulates systemic and local immune responses.

Gut bacteria like lactobacilli can also metabolise tryptophan, resulting in bioactive indole derivatives which stimulate locally the production of IL22 via the aryl hydrocarbon receptor (AhR). The cytokine IL22, which is produced by different immune cells, promotes the microbial symbiosis excluding yeasts, and protects the gut mucosa from inflammation<sup>1</sup>.

Only 1% of tryptophan is being metabolised into serotonin via a separate metabolic pathway. This serotonin pathway takes place mainly in the gut and promotes peristaltic movement and secretory functions of the intestine<sup>2</sup>.



In chronic states of inflammation like IBD (inflammatory bowel disease), pro-inflammatory cytokines including IFN- $\gamma$ , TNF- $\alpha$ , IL1 $\beta$ , IL2, IL6, IL18, and IFN- $\alpha$  induce the expression of IDO1. This activates the kynurenine pathway and results in low tryptophan and high kynurenine levels in stool (*Fig. 2a*). The tryptophan levels correlate inversely with the inflammatory activity in the gut. They are markedly lower in active Crohn's disease and active ulcerative colitis compared to controls or to inactive disease (*Fig. 2b*). The tryptophan levels of IBD patients could be normalised by successful therapy using the biological infliximab, a TNF- $\alpha$  inhibitor, but not using vedolizumab, an integrin inhibitor<sup>2</sup>.



*Fig. 2:* Tryptophan deficiency in IBD patients. (a) Tryptophan in stool is decreased in IBD patients. (b) Patients with active Crohn's disease or ulcerative colitis have lower serum tryptophan levels than patients in remission or healthy controls. *HS*, healthy subjects; *CD*, Crohn's disease; *UC*, ulcerative colitis. Adapted from Lamas et al. (2016)<sup>4</sup> and Nikolaus et al. (2017)<sup>2</sup>.

The levels of various tryptophan metabolites deriving from kynurenine via different pathways are also significantly altered in IBD. While quinolinic acid is significantly higher in Crohn's disease and ulcerative colitis, only in Crohn's disease kynurenic acid is found substantially less than in healthy controls (*Fig. 3*). Within the kynurenine pathway, kynurenic acid plays a regulatory role by inhibiting quinolinic acid. This mechanism seems to be ineffective in active IBD<sup>2</sup>.

Tryptophan deficiency could contribute to development of IBD or aggravate the activity of the inflammation (*Fig. 4*). An indication for this is that low tryptophan levels have been demonstrated in IBD patients<sup>2, 4</sup>. In a mouse model, tryptophan deficiency caused increased susceptibility for DSS-induced colitis, while tryptophan supplementation had anti-inflammatory effects<sup>5</sup>.



*Fig. 3:* The levels of the tryptophan metabolites quinolinic acid and kynurenic acid are altered in IBD patients. (a) Elevated serum levels of quinolinic acid can be detected in Crohn's disease and ulcerative colitis. (b) Kynurenic acid is lower in Crohn's disease but not in ulcerative colitis. *QuinA*, quinolinic acid; *KynA*, kynurenic acid. Adapted from Nikolaus et al. (2017)<sup>2</sup>. With normal IDO1 activity, the tryptophan metabolites have a protective function against inflammation and stabilise the intestinal immune system in mice, while high IDO1 activity due to inflammation reduces the benefits of these metabolites<sup>1</sup>.



*Fig. 4:* Activation of IDO1, e.g. due to inflammation, changes the equilibrium between tryptophan and its metabolites. Tryptophan levels decrease while more quinolinic acid and less kynurenic acid and indole derivatives are produced. *IDO1*, indoleamine 2,3-dioxygenase 1; *QuinA*, quinolinic acid; *KynA*, kynurenic acid; *IndolAld*, indole derivate.

<i>IDK®</i> <b>Tryptophan</b>	
Matrix	Stool
Sample volume	15 mg
Test principle	ELISA
Cat. No.	K 7729

#### Also available:

IDK<sup>®</sup> Tryptophan ELISA (serum, plasma, urine, dried blood samples) (K 7730) IDK<sup>®</sup> Tryptophan high sensitive ELISA (KR3730) *(for research use only)* IDK<sup>®</sup> Serotonin ELISA (serum, dried blood samples) (K 6880) IDK<sup>®</sup> Kynurenic acid (KynA) (K 7735)

### CE

US: all products: Research Use Only. Not for use in diagnostic procedures.

#### Literature:

- <sup>1</sup> Zelante T, Iannitti RG, Cunha C, De Luca A, Giovannini G, Pieraccini G, Zecchi R, D'Angelo C, Massi-Benedetti C, Fallarino F, Carvalho A, Puccetti P & Romani L (2013). Tryptophan Catabolites from Microbiota Engage Aryl Hydrocarbon Receptor and Balance Mucosal Reactivity via Interleukin-22. *Immunity* 39: 372–385.
- <sup>2</sup> Nikolaus S, Schulte B, Al-Massad N, Thieme F, Schulte DM, Bethge J, Rehman A, Tran F, Aden K, Häsler R, Moll N, Schütze G, Schwarz MJ, Waetzig GH, Rosenstiel P, Krawczak M, Szymczak S & Schreiber S (2017). Increased Tryptophan Metabolism Is Associated With Activity of Inflammatory Bowel Diseases. *Gastroenterology* 153: 1504–1516.
- <sup>3</sup> Thorburn AN, Macia L & Mackay CR (2014). Diet, Metabolites, and "Western-Lifestyle" Inflammatory Diseases. Immunity 40: 833–842.
- <sup>4</sup> Lamas B, Richard ML, Leducq V, Pham HP, Michel ML, Da Costa G, Bridonneau C, Jegou S, Hoffmann TW, Natividad JM, Brot L, Taleb S, Couturier-Maillard A, Nion-Larmurier I, Merabtene F, Seksik P, Bourrier A, Cosnes J, Ryffel B, Beaugerie L, et al (2016). CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. *Nat Med* 22: 598–605.
- <sup>5</sup> Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, Sigl V, Hanada T, Hanada R, Lipinski S, Wild B, Camargo SMR, Singer D, Richter A, Kuba K, Fukamizu A, Schreiber S, Clevers H, Verrey F, Rosenstiel P, Penninger JM (2012). ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* 487: 477–481.

Immundiagnostik also offers further PCR diagnostic for microbiome and colon (FOMB 7)





Distribuito in ITALIA da Li StarFish S.r.I. Via Cavour, 35 20063 Cernusco S/N (MI) telefono 02-92150794 info@listarfish.it www.listarfish.it

