Discover. Develop. Apply.

A Guide to the IDK® Vitamin D + IBD Testing Panel

Immundiagnostik, Inc.

Overview

Tools for the investigation of IBD and vitamin D

IBD

- Calprotectin ELISA
- Pancreatic Elastase ELISA
- Zonulin ELISA
- sIgA ELISA
- α1 - Antitrypsin ELISA
- Lysozyme ELISA
- Myeloperoxidase (MPO) ELISA

Vitamin D

- 1,25 $(OH)_2$ Vitamin D ELISA
- 1,25$(OH)_2$ Vitamin D3/2 ImmuTube® LC-MS/MS Kit
- 1,25$(OH)_2$ Vitamin D3/2 LC-MS/MS SIurry

About the Panel

IBD and vitamin D deficiency are highly prevalent health issues worldwide and in the US, especially. Studies have connected the two, showing that vitamin D deficiencies can increase the risk of developing IBD. It has also been found that many individuals with IBD have a higher prevalence of vitamin D deficiency. It is suspected that the gut microbiome is responsible for metabolizing vitamin D from its precursor form into its active form. Thus, a more diverse gut microbiome will allow for increased metabolism and, subsequently, more active vitamin D.

This connection between IBD and vitamin D suggests that any labs testing for IBD should consider adding vitamin D tests to their portfolio to help better evaluate the overall health of individuals affected by IBD.

Immundiagnostik, Inc. offers tools to investigate both IBD and vitamin D to create the IDK® IBD + Vitamin D Testing Panel.

Calprotectin ELISA

Description, Indication and Features

Description

Calprotectin shows high stability in feces and has been established as a fecal marker of inflammatory bowel diseases (IBD) because it is released into the stool by the intestinal mucosa in response to intestinal inflammation.

Indication

The IDK® Calprotectin ELISA is an enzyme immunoassay intended for the quantitative determination of calprotectin (MRP 8/14, S100A8/A9) in stool.

Features

- Sample Type: Stool
- Sample Volume: 15 mg
- Incubation Time: 1h 10m
- Standard Range: 13-840 ng/ml
- Compatible with the IDK Extract® Stool Sample Preparation System

The IDK® Calprotectin ELISA is For Research Use Only in the U.S. Not for Use in Diagnostic Procedures.
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Calprotectin ELISA

Assay Principle

The IDK® Calprotectin ELISA Kit is designed, developed, and produced for the quantitative measurement of human calprotectin in stool samples. The Calprotectin ELISA utilizes the two-site "sandwich" technique with two selected antibodies that bind to different epitopes of human calprotectin.

Assay standards, controls, and samples are added directly to wells of a microtiter plate coated with antibodies to calprotectin. After a short incubation period, the plate is washed and horseradish peroxidase (HRP) conjugated human calprotectin-specific monoclonal antibody is added to each well. After the second incubation period, a "sandwich" of solid-phase antibody – human calprotectin – HRP conjugated monoclonal antibody" is formed.

The unbound monoclonal antibodies and buffer matrix are removed in the subsequent washing step. For the detection of this immunocomplex, the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the wall of each microtiter well is directly proportional to the amount of human calprotectin in the test sample.

A standard curve is generated by plotting the absorbance versus the respective human calprotectin concentration for each standard on a point-to-point or 4-parameter curve fitting. The concentration of fecal human calprotectin in test samples is determined directly from this standard curve of the Calprotectin ELISA.

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Pancreatic Elastase ELISA

Description, Indication and Features

Description

Pancreatic elastase is a proteolytic enzyme produced in the pancreatic acinar cells and then secreted into the duodenum where it is converted into an active enzyme. Upon activation, pancreatic elastase degrades proteins and plays an important role in digestion. The stool concentration of pancreatic elastase reflects the secretory capacity of the pancreas, allowing for the investigation of exocrine pancreatic insufficiency (EPI). EPI is characterized by reduced pancreatic enzyme activity which inhibits digestion.

Indication

The IDK® Pancreatic Elastase ELISA is an enzyme immunoassay intended for the quantitative determination of human pancreatic elastase in stool.

Features

- Sample Type: Stool
- Sample Volume: 15 mg
- Incubation Time: 1h 10m
- Standard Range: 2-72 ng/ml
- Compatible with the IDK Extract® Stool Sample Preparation System

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Pancreatic Elastase ELISA

Assay Principle

The IDK® Pancreatic Elastase ELISA is intended for the quantitative determination of pancreatic elastase in stool.

In the first incubation step, the pancreatic elastase in the samples is bound to monoclonal antibodies and immobilized to the surface of the microtiter wells. To remove all unbound substances, a washing step is carried out. In a second incubation step, a peroxidase-labeled conjugate (mouse anti-pancreatic elastase) is added which recognizes specifically the bound pancreatic elastase.

After another washing step to remove all unbound substances, the solid phase is incubated with the substrate, tetramethyl-benzidine (TMB), which reacts with the peroxidase. An acidic stop solution is added to stop the reaction. The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of pancreatic elastase.

A dose-response curve of absorbance unit (optical density, OD at 450nm) vs. concentration is generated using the values obtained from the standards. Pancreatic elastase, present in the stool samples, is determined directly from this curve

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Zonulin ELISA

Description, Indication and Features

Description

Zonulin is a tight-junction regulating protein in the digestive tract. Zonulin binds to a specific receptor on the surface of intestinal epithelia and triggers a cascade of biochemical events which induces tight junction disassembly and a subsequent permeability increase of the intestinal epithelia, allowing some substances to pass through and activate immune reactions.

Increased intestinal permeability, also known as 'leaky gut', is associated with metabolic syndrome, obesity, and several autoimmune, inflammatory, and neoplastic diseases. Based on evidence, leaky gut plays a meaningful role in diseases such as multiple sclerosis, rheumatoid arthritis, asthma, and inflammatory bowel diseases.

Indication

The IDK® Zonulin ELISA is intended for the quantitative determination of zonulin family peptides (ZFP) in stool.

Features

- Sample Type: Stool
- Sample Volume: 15 mg
- Incubation Time: 2h 10m
- Standard Range: 0.25-64 ng/ml
- Also available for use with serum (KR 5601)

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Zonulin ELISA

Assay Principle

The IDK® Zonulin ELISA is an assay based on the method of competitive ELISA. As a first preparation step, biotinylated zonulin family peptides (ZFP) are added to the samples, standards, and controls. Afterward, aliquots of the treated samples, standards, and controls are transferred and incubated in microtiter plate wells coated with polyclonal anti-ZFP antibodies.

During the incubation, the free target antigen in the samples competes with the biotinylated ZFP for the binding of the polyclonal anti-ZFP antibodies immobilized on the microtiter plate wells. The unbound components are removed by a washing step.

During a second incubation step, peroxidase-labeled streptavidin, which binds to the biotinylated ZFP, is added to each microtiter well. After a washing step to remove the unbound components, the peroxidase substrate tetramethylbenzidine is added. Finally, the enzymatic reaction is terminated by an acidic stop solution.

The color changes from blue to yellow and the absorbance is measured in the photometer at 450 nm. The intensity of the yellow color is inversely proportional to the ZFP concentration in the sample; this means high ZFP concentration in the sample reduces the concentration of the biotinylated ZFP bound to the immobilized anti-ZFP antibodies and lowers the photometric signal.

A dose-response curve of absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from the standard.

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sIgA ELISA

Description, Indication and Features

Description

Fecal secretory IgA (sIgA) is the most abundant class of antibodies found in the intestinal lumen. The immune tolerance of the intestinal mucosa can be evaluated using the concentration of fecal sIgA. A deficiency of sIgA points to a diminished activity of the mucosa immune system; whereas increased sIgA values indicate increased activity and a local inflammation of the intestinal mucosa.

Indication

The IDK® sIgA ELISA is intended for the quantitative determination of secretory IgA in saliva and stool.

Features

- Sample Type: Saliva, Stool
- Sample Volume: 10 µl Saliva, 15 mg Stool
- Incubation Time: 2h 20m
- Standard Range: 22.2-600 ng/ml
- Compatible with the IDK Extract® Stool Sample Preparation System

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sIgA ELISA

Assay Principle

The IDK® sIgA ELISA is intended for the quantitative determination of secretory IgA in stool and saliva.

In the first incubation step, the sIgA in the samples is bound to polyclonal antibodies (rabbit anti-human IgA), which are immobilized to the surface of the microtiter wells. To remove all unbound substances, a washing step is carried out. In a second incubation step, a peroxidase-labeled conjugate (mouse anti-sIgA) is added which recognizes specifically the bound secretory IgA. After another washing step to remove all unbound substances, the solid phase is incubated with the substrate, tetramethylbenzidine (TMB).

An acidic stop solution is then added to stop the reaction. The color converts from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of secretory IgA. A dose-response curve of the absorbance unit (optical density, OD) vs. concentration is generated using the results obtained from the standards.

Secretory IgA, present in the samples, is determined directly from this curve.

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α1-Antitrypsin ELISA

Description, Indication and Features

Description

α1-Antitrypsin is released during inflammatory processes by polymorphonuclear neutrophilic granulocytes (PMN) to reduce the proteolytic activity of PMN elastase in the inflammation region. α1-Antitrypsin is also a serine proteinase inhibitor, thus it acts as both a regulatory and anti-inflammatory protein.

Indication

The IDK® α1-Antitrypsin ELISA is an enzyme immunoassay intended for the quantitative determination of alpha-1-antitrypsin in stool.

Features

- Sample Type: Stool
- Sample Volume: 15 mg
- Incubation Time: 2h 15m
- Standard Range: 3.3-90 µg/l
- Compatible with the IDK Extract® Stool Sample Preparation System

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α1-Antitrypsin ELISA

Assay Principle

The IDK® α1-Antitrypsin ELISA is designed for the quantitative determination of α1-antitrypsin in stool.

The assay utilizes the sandwich technique with two selected antibodies that bind to human α1-antitrypsin. Standards, controls, and prediluted samples assayed for human α1-antitrypsin are added into the wells of a microplate coated with a high affine anti-human α1-antitrypsin antibody.

During the first incubation step, α1-antitrypsin is bound by the immobilized antibody. Then a peroxidase-conjugated polyclonal anti-human α1-antitrypsin antibody is added into each microtiter well and a sandwich of capture antibody – human α1-antitrypsin – peroxidase-conjugate is formed. Tetramethylbenzidine is used as peroxidase substrate. Finally, an acidic stop solution is added to terminate the reaction.

The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of α1-antitrypsin. A dose-response curve of the absorbance unit (optical density, OD at 450nm) vs. concentration is generated using the values obtained from the standard. The presence of α1-antitrypsin in the samples is determined directly from this curve.

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Lysozyme ELISA

Description, Indication and Features

Description

Lysozyme is an antimicrobial enzyme that is part of the immune system and is detected in all cells of the inflammatory infiltrate during an acute flare of Crohn's disease. To some extent, lysozyme is also secreted actively by mononuclear cells into the bowel lumen.

Indication

The IDK® Lysozyme ELISA is an enzyme immunoassay intended for the quantitative determination of lysozyme in stool.

Features

- Sample Type: Stool
- Sample Volume: 15 mg
- Incubation Time: 2h 20m
- Standard Range: 1.1-30 ng/ml
- Compatible with the IDK Extract® Stool Sample Preparation System

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Lysozyme ELISA

Assay Principle

The IDK® Lysozyme ELISA utilizes the “sandwich” technique with two selected antibodies that recognize human lysozyme. Standards, controls, and diluted samples assayed for human lysozyme are added into the wells of a microplate coated with a high affine anti-human lysozyme antibody.

During the first incubation step, lysozyme is bound by the immobilized antibody. Then a peroxidase-conjugated anti-human lysozyme antibody is added into each microtiter well and a “sandwich” of capture antibody - human lysozyme – peroxidase-conjugate is formed. Tetramethylbenzidine is used as peroxidase substrate. Finally, an acidic stop solution is added to terminate the enzymatic reaction.

The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of lysozyme. A dose-response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from the standards. The presence of lysozyme in the samples is determined directly from this curve.

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Myeloperoxidase (MPO) ELISA

Description, Indication and Features

Description

Myeloperoxidase (MPO) is a hemoprotein secreted by neutrophils upon their activation. The determination of MPO in stool, therefore, can help in the investigation of the inflammatory activity of Crohn's disease or ulcerative colitis.

Indication

The IDK® MPO ELISA is an enzyme immunoassay for the quantitative determination of myeloperoxidase in stool and urine.

Features

- Sample Type: Urine, Stool
- Sample Size: 100 µl Urine, 100 mg Stool
- Incubation Time: 2h 10m
- Standard Range: 1.9-30 ng/ml

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Myeloperoxidase (MPO) ELISA

Assay Principle

The IDK® MPO ELISA is designed for the quantitative determination of myeloperoxidase (MPO) in urine and stool.

In the first incubation step, the myeloperoxidase in the samples is bound to an available excess of antibodies against myeloperoxidase, which are immobilized to the surface of the microtiter plates. To remove all unbound substances, a washing step is carried out. In a second incubation step, a peroxidase-labeled antibody against MPO is added. After another washing step to remove all unbound substances, the solid phase is incubated with the substrate, tetramethylbenzidine (TMB). An acidic stop solution is then added to stop the reaction.

The color converts from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of MPO in the sample. A dose-response curve of the absorbance unit (optical density, OD) vs. concentration is generated using results obtained from the calibrators. The presence of MPO in the samples is determined directly from this curve.

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1,25 $(OH)_2$ Vitamin D ELISA

Description, Indication and Features

Description

25-OH vitamin D is metabolized in the liver into 1,25 $(OH)_2$ vitamin D. A deficiency of 1,25 $(OH)_2$ vitamin D can be explained by metabolic disturbances, caused either by genetic enzyme defects (rare) or kidney malfunctions (more common). Even slightly impaired kidney function can lead to a decrease of 1,25 $(OH)_2$ vitamin D concentration.

Indication

The IDK® 1,25 $(OH)_2$ Vitamin D ELISA Kit is intended for the quantitative determination of 1,25-dihydroxy vitamin D in serum and plasma.

Features

- Sample Type: Serum, Plasma
- Sample Volume: 1 ml
- Incubation Time: 19h 15m
- Standard Range: 5.1-200 pg/ml

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1,25 $(OH)_2$ Vitamin D ELISA

Assay Principle

The IDK® 1,25$(OH)_2$ Vitamin D ELISA utilizes a competitive enzyme immunoassay (EIA) technique with a selected monoclonal antibody recognizing 1,25-dihydroxy vitamin D.

Standards, controls, and samples assayed for 1,25-dihydroxy vitamin D are incubated after the extraction step with the detection antibody. The pre-incubated solution is then transferred to the microplate coated with 1,25-dihydroxy vitamin D. During this incubation step, 1,25-dihydroxy vitamin D in the sample and a fixed amount of 1,25-dihydroxy vitamin D bound to the microtiter well compete for the binding of the detection antibodies. Then a peroxidase-conjugated anti-mouse antibody is added to each microplate well and a complex of 1,25-dihydroxy vitamin D – detection antibody – peroxidase conjugate is formed. Tetramethylbenzidine (TMB) is used as a peroxidase substrate.

Finally, an acidic stop solution is added to terminate the reaction, whereby the color changes from blue to yellow. The intensity of the yellow color is inversely proportional to the concentration of 1,25-dihydroxy vitamin D. A dose-response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from the standard. The presence of 1,25-dihydroxy vitamin D in the samples is determined from this curve.

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$1,25(OH)_2$ Vitamin D3/2 Immutube® LC-MS/MS Kit

Description, Indication and Features

Description

Supplemental vitamin D is available in two distinct forms: ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). Pharmacopoeias have officially regarded these two forms as equivalent and interchangeable based on studies of rickets prevention in infants. The determination of 1,25 dihydroxy vitamin D3/D2 as a measure of 1,25 dihydroxy vitamin D status provides an objective, quantitative measure of the biological response to vitamin D administration.

Indication

The IDK® $1,25(OH)_2$Vitamin D3/2 ImmuTube® LC-MS/MS Kit is for the quantification of $1,25-(OH)_2$Vitamin D3/D2 in serum and plasma.

Features

- Sample Type: Serum, Plasma
- Sample Volume: 500 µl

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$1,25(OH)_2$ Vitamin D3/2 LC-MS/MS Slurry

Description, Indication and Features

Description

After the sample extraction process, the slurry is briefly added to the serum for a short period of incubation to prepare it for the LC-MS/MS testing device.

Indication

The IDK® $1,25(OH)_2$ Vitamin D Slurry is intended to be used in conjunction with the IDK® $1,25(OH)_2$ Vitamin D3/2 ImmuTube® LC-MS/MS Kit.

Features

- Sample Type: Plasma, Serum
- Sample Volume: 500 µl
- Size: 100 mL

The IDK® 1,25(OH)2 Vitamin D3/2 LC-MS/MS Slurry is for Research Use Only in the U.S.
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IBD

Pancreatic Elastase ELISA

Calprotectin ELISA

Lysozyme ELISA

Myeloperoxidase (MPO) ELISA

Zonulin ELISA

sIgA ELISA

α1-Antitrypsin ELISA

Vitamin D

1,25(OH)$_2$ Vitamin D ELISA

1,25(OH)$_2$ Vitamin D3/2 Immutube® LC-MS/MS Kit

1,25(OH)$_2$ Vitamin D3/2 LC-MS/MS Slurry

The Panel

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Conclusion

Several studies have demonstrated a clear connection between vitamin D deficiency and the presence of IBD. Vitamin D deficiencies can lead to an increased risk of developing IBD and many individuals with IBD also have a higher prevalence of vitamin D deficiency. These findings indicate the gut microbiome may be involved in metabolizing vitamin D into its active form such that a more diverse gut microbiome is related to an increased rate of metabolism.

Immundiagnostik, Inc. offers a full 'Vitamin D + IBD panel' of immunoassays which allow labs to better evaluate the overall health of individuals affected by IBD. This panel combines our most popular gastrointestinal ELISAs, which monitor intestinal inflammation and permeability, with our vitamin D kits, which evaluate active and precursor vitamin D supply.

Contact us for more information about adding vitamin D to your lab's IBD testing panel.

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