iVYCHECK GIP Stool

For research use only

KT-6410R: Rapid test for the detection of Gluten immunogenic peptides (GIP) in stool samples, 10 Tests.
KT-5737R: Rapid test for the detection of Gluten immunogenic peptides (GIP) in stool samples, 25 Tests.

LIST OF PRODUCT VARIANTS

Product	Contents	REF
iVYCHECK GIP Stool, 10T	10 test	KT-6410R
iVYCHECK GIP Stool, 25T	25 test	KT-5737R

INTRODUCTION

Celiac disease is a chronic disorder of autoimmune origin that is characterized by a permanent intolerance to the proteins of gluten.

It appears in genetically predisposed people, both adults and children. It has an prevalence in the population of above 1:100, most frequently in children. The disease affects the small intestine and provokes the atrophy of the intestinal villi and thereby interferes with the absorption of nutrients such as proteins, fats, carbohydrates, salts and vitamins. It doesn't only affect the intestine but is in fact a systemic disease which can cause lesions in the skin, joints and other organs.

The strict adherence to the Gluten Free Diet (GFD) is essential for reducing those symptoms of the disease, avoiding nutritional deficiencies and improving the quality of life. However, based on multiple different studies, dietary transgressions, whether voluntary or involuntary, are frequent (32.6-55.4%) in celiac patients, especially in adults.

At present, there is no direct method for the monitoring the adherence to the GFD. Gluten immunogenic peptides (GIP) are fragments of gluten proteins which are resistant to gastrointestinal digestion and trigger immune responses in celiac patients. The recuperation of measurable quantities of GIP in stool indicates that a patient has consumed gluten. The present of GIP in the stools constitutes a precise, direct marker for the control of the GFD in the long and short term.

INTENDED USE AND CHARACTERISTICS

Indication	Sanitary product
Function	Detection of the presence of GIP in stool samples
Target	Monitoring of the GFD (celiac disease)
Method Type	Manual
Analysis Type	Qualitative
Sample Type	Stool
Testing population	Celiac patients, patients with non-celiac wheat sensitivity, gluten-allergy or other disorders related to gluten.
Intended Use	iVYCHECK GIP Stool is intended for professional use for the qualitative monitoring of the gluten-free diet via the detection of GIP in stool samples from celiac patients or patients with other gluten-related disorders.
Intended User	For research use
Assay Type	Rapid immunochromatographic test

TECHNICAL SPECIFICATIONS

Sample quantity	1 gram
Diagnostic sensitivity *	94,6% (IC95:86-100%)
Diagnostic specificity *	100% (IC95:98,8-100%)
Positive predictive value *	100% (IC95:98,6-100%)
Negative predictive value *	95,2% (IC95:87,6-100%)
Reproducibility	98% (IC95: 96-99%)
Repeatability	98% (IC95: 94-100%)
Lower detection limit **	0,3 µg GIP***/g of stool

Upper detection limit	≥1 mg GIP***/g of stool (supraphysiological concentration)

** Associated with people without restrictions on gluten consumption in the diet. Intake not controlled.*
*** It is defined as the value where 95% of the samples at that concentration have a positive result.*
**** Based on the 33-mer peptide of α-gliadin.*

INTERFERENCES

No interference detected to date.

KIT CONTENTS

References	Description	KT-6410R	KT-5737R
RS-6527R	Stick Dilution Solution_iVYC	10 uds	25 uds
RS-6514R	iVYCHECK Stick (with plastic pipette)	10 uds	25 uds
RS-6529R	Extraction Solution_iVYC*	40 mL	40 mL
RS-6515R	Extraction Tube_iVYC	10 uds	25 uds

*NOTE: For the preparation of the reagents, consult the protocol.

Non-provided, required materials:
Sample collection pot, 100µl-1ml pipette and disposable tips, plastic vials Eppendorf®, powder-free nitrile gloves, laboratory coat, (96-100%) ethanol.

SECURITY INFORMATION

The non-included ethanol (96-100%) is classified, according to Regulation (CE) 1272/2008 (CLP) as a category 2 reagent due to two risk classes:

2.6. Flammable liquids (H225 Highly flammable liquids and vapours)
3.3. Serious injuries or ocular irritation (H319 Causes serious eye irritation)

Follow the indications from the safety data sheet of the provider.

STORAGE AND TRANSPORT CONDITIONS

Storage conditions	15° a 30° / 59°F a 86°F
Transport conditions	Room temperature
Stability in use *	3 months

* Once the reagents have been prepared and aliquoted as indicated in the procedure.

STABILITY

The product is stable until the expiration date indicated on the label provided that the storage conditions indicated on the kit are maintained.

RECOMMENDATIONS, PRECAUTIONS AND LIMITATIONS

Any serious incident which occurs related to the use of the kit should be communicated to the manufacturer and the relevant authority.

- This kit has been designed for research use.
- Use the appropriate PPE (disposable protective gloves, laboratory coat) when working with kit reagents and samples.
- The kit should be used at standard room temperature conditions.
- Close the bottle and vial that contain the components of the kit immediately after use and do not interchange lids.
- Do no combine no exchange reagents between kits.
- Once the lateral flow device has been removed from the sealed metallic pouch, it must be used as soon as possible in strict clean conditions.
- Dispose of the unused reagents and waste material in accordance with the relevant regulation.
- It is important to use the indicate amount of sample. The usage of more or less than recommended could result in erroneous results.

- Homogenise the sample well. An insufficient homogenisation could cause variability in the replicates of a sample.
- In the case of excessively liquid or hard stools, the use of a sample of the subsequent deposit (where possible) is recommended.
- Pay attention to the mixing process. A weak or shortened mixing process could lead to false negatives.
- The usage of this kit should comply with the safety documentation provided.
- The avoidance between clinical samples can be avoided solely by the application of good laboratory practise and the careful compliance with the protocol provided in the instructions for use. All reagents should be monitored for impurities and contaminations. Any reagent with suspected issues should be disposed of.
- Control should be taken during the management of clinical material. Incorrect manipulation of samples could result in a contamination and/or deterioration of components of the kit. BIOMEDAL S.L. will not be responsible for the incorrect usage of the kit.
- Alcohol based disinfectants can damage the labels on the bottles and vials.
- Poor vision, colour-blindness or poor illumination can affect the capacity to correctly interpret the kit.

Procedural Limitations

- Read all of the instructions thoroughly before employing the kit.
- Adequate systems for collection, transportation, storage and processing of samples are required for the optimal usage of the product.
- Do not use the kit after the expiry date.

Clinical Limitations

- This kit is not valid for the analysis of neonatal samples.

COLLECTION AND STORAGE OF SAMPLES

Sample collection recommendations:

• If gluten ingestion is suspected, please consider the following information:

Maximum detection time from ingestion	1-6 days
Optimal detection time from ingestion*	1-2 days
Minimum amount of detectable gluten	50mg

**To maximize the probability of detection of the kit, sampling is recommended 1-2 days after gluten intake.*

At least 1-2 g or mL (for liquid samples) of stool should be collected. These samples must be collected in a clean, dry container without any added chemical additives or preservatives. The sample should be prevented from coming into contact with urine or toilet water.

For a follow-up of the gluten-free diet, it is recommended to collect two stools samples a week (for example: Monday and Thursday).

Storage of stool samples:

Storage at room temperature	Maximum time 72 hours
Storage at 4°C	Maximum time 72 hours
Storage at -20°C	Maximum time 24 months
Freeze/Thaw Cycles	Maximum six cycles

PROTOCOL

PREPARATION OF REAGENTS

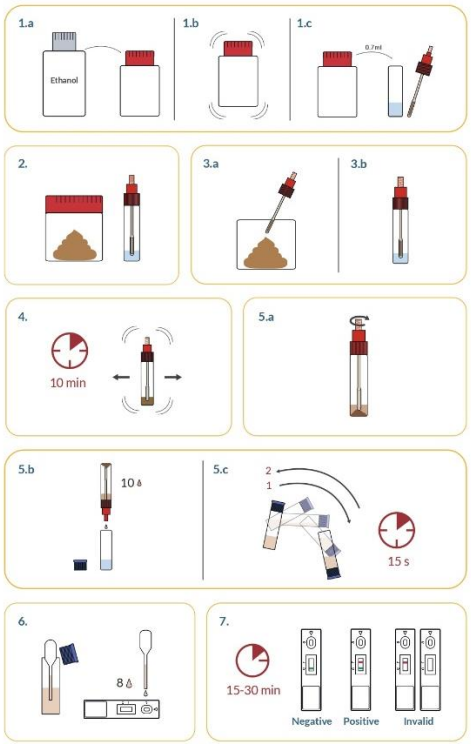
The extraction solution (Ref.: RS-6529) should be prepared previous to the initiation of the assay. Add 60ml of ethanol absolute (100%) or 62.5ml of 96% ethanol to bottle RS-6529 (Extraction Solution) and mix well. Once the ethanol is added, mark the relevant box on the label as a remind of its addition. Finally, add 0.7ml of this solution to each extraction tube (RS-6515_ Extraction Tube_iVYC).

PROTOCOL:

1. Defrost any frozen simples. Samples should be collected following the indicated methodology. The sample must be completely defrosted before its analysis.
2. Use a clean pipette tip to move and homogenise the stool samples.
3. Take a sample of the stool using the small spoon attached to the red lid of the tube (to which extraction solution was previously added). The correct amount of stool is a ´level spoonful´ of sample.
4. Reintroduce the spoon with the sample into the tube and agitate until the sample is fully disconnects from the spoon.
5. Close the tube and shake vigorously and intermittently for 10 minutes. A vortex-type shaker can be used for this stage.
6. Unscrew the white lid of the red tube and transfer exactly 10 drops to the tube with the blue lid, which contains the dilution solution. Mix via inversion for 15 seconds.
7. Open the metallic pouch, which contains the lateral flow device, and use the contained pipette to transfer 8 drops or transfer 100µl using a Manual Pipette of the solution from stage 6 to the hole in the lateral flow device marked S (sample).
8. Make a visual assessment of the result from the lateral flow device after 15 minutes (a positive result will be observed for samples containing GIP peptide above the limit of detection). In the case of now observing a red line after 15 minutes, wait until 30 minutes as, in some cases, low levels of GIP require a longer time to result in a positive result

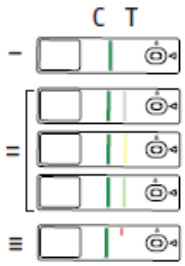
   Do not read the result after more than 30 minutes

DIAGRAMATIC PROTOCOL



RESULT INTERPRETATION

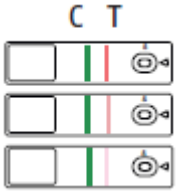
- **NEGATIVE**



I. A single green line appears in the Control Zone (C). The intensity of the line Control (C) is not relevant.
II. If a line appears in the Test Zone (T) of a color other than red (eg grey, yellow, green) and a green line
appears in the Control Zone (C).
III. If the red line is not complete (eg red dots appear in the T zone) and a green line appears in the
Control Zone (C).

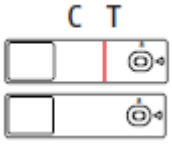
No gluten has been detected in the stool sample.

- **POSITIVE**



If a GREEN line in the Control Zone (C) is visible next to a red line in the Test Zone (T), independently of
the intensity obtained; the test result is positive.
If you have obtained a positive result, there is a high probability that there has been a gluten ingestion 1-6 days before performing the test. Check the food eaten to identify the origin of the gluten.

- **INVALID**



If the GREEN line does not appear, the test has not worked correctly and the results are invalid. Review the instructions and perform the test again following the instructions.

INTERNAL QUALITY CONTROL

Each batch is subject to an internal quality control before its release for sale. The quality control is performed using real positive and negative samples as well as stool samples spiked with GIP.

The following acceptance criteria are applied:

- ✓ The tested samples should give the anticipated result
- ✓ The samples spiked to the Limit of Detection should give a positive result.

SYMBOLS

RUO Research Use Only	Consult instructions for use
Manufacturer	Keep dry
REF Catalogue number	Caution
LOT Batch code	Temperature limitation
Use by (expiration date)	
Contains sufficient for <n> tests	

REFERENCES

- Comino I., et al.; "Monitoring of gluten-free diet compliance in celiac patients by assessment of gliadin 33-mer equivalent epitopes in feces"; The American journal of clinical nutrition; 2012; 95: 670-677.
- Silvester J., et al.: "Celiac Disease Patients Should No Longer Be Consuming Measurable Amounts of Gluten"; Gastroenterology; 2020; 158: 1497-1499.
- Coto L, Sousa C, Cebolla A. "Individual variability in patterns and dynamics of fecal gluten immunogenic peptides excretion after low gluten intake". Eur J Nutr. 2022 Jun;61(4):2033-2049.

MODIFICATIONS IN THE LAST VERSION

M-167R

Ed. 01. Revised: 20 October 2022

For more information, please visit our website or contract us: info@biomedal.com