# EUROLINE-FOOD

## EUROLINE-FOOD Profile 216 (IgG)
## Test instruction

| ORDER NO. | ANTIBODIES AGAINST | IG CLASS | SUBSTRATE | FORMAT |
|---|---|---|---|---|
| DP 3024-0404-1 G | Foods | IgG | Extract-coated membrane strips | 4 x 04 (16) |

**Indication:** The EUROLINE-FOOD test supports the diagnosis of sensitisations against foods and food additives, which may lead to unspecific health problems, by detection of IgG antibodies (e.g. gastrointestinal disorders, inflammatory skin diseases, migraine, chronic fatigue syndrome, etc.).

**Application:** The EUROLINE-FOOD test provides semiquantitative in vitro determination of the food-specific immunoglobulin class IgG in serum or plasma and contributes to the diagnosis of intolerances against foods and food additives. The test is a multi-parameter test which contains optimised combinations of relevant foods and their respective additives and therefore enables simultaneous analyses of IgG against both.

**Test principle:** The test kit contains test strips each coated with 54 different foods and food additives. The test strips are first moistened and then incubated with patient sample in the first reaction step. If samples contain specific antibodies of class IgG (and IgE, if present), they will bind to the antigenic components coated on the strip. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

**Contents of a test kit:**

| Description | Format | Symbol |
|---|---|---|
| 1. **Test strip 1 coated with 54 foods and food additives and calibrators** | 1 x 4 strips | STRIP1 |
| 2. **Test strip 2 coated with 54 foods and food additives and calibrators** | 1 x 4 strips | STRIP2 |
| 3. **Test strip 3 coated with 54 foods and food additives and calibrators** | 1 x 4 strips | STRIP3 |
| 4. **Test strip 4 coated with 54 foods and food additives and calibrators** | 1 x 4 strips | STRIP4 |
| 5. **Enzyme conjugate** Alkaline phosphatase-labelled anti-human IgG (goat), ready for use | 1 x 30 ml | CONJUGATE |
| 6. **Universal buffer** 10x concentrated | 1 x 100 ml | BUFFER 10x |
| 7. **Substrate solution** Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use | 1 x 30 ml | SUBSTRATE |
| 8. **Incubation tray,** volume-reduced (400 µl) | 2 x 10 channels | TRAY |
| 9. **Plastic foil** | 1 sheet | |
| 10. **Instruction booklet** | 1 booklet | |

| | | | |
|---|---|---|---|
| LOT | Lot description | | Storage temperature |
| IVD | In vitro diagnostic medical device | CE | Unopened usable until |

Performance of the test requires incubation trays or other components, which are not provided in the test kits. They are available from EUROIMMUN under the following order numbers:
ZD 9897-0130 Incubation tray (volume-reduced 400 µl) with 30 channels (black, compatible with EUROBlotMaster and EUROBlotCamera System)
ZD 9897-0144 Incubation tray (volume-reduced 400 µl) with 44 channels (black, compatible with EUROBlotOne, EUROBlotMaster and EUROBlotCamera System)

**Modifications to the former version are marked in grey.**

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For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** you require green paper and adhesive plastic foil:
ZD 9880-0101 Green paper (1 sheet)
ZD 9885-0116 Adhesive foil for approx. 16 test strips
ZD 9885-0130 Adhesive foil for approx. 30 test strips
For covering the incubation trays the adhesive foil can be used as well.

## Preparation and stability of the reagents

**Note:** All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. Unopened, reagents are stable until the indicated expiry date when stored at +2°C to +8°C. After initial opening, reagents are stable for 12 months or until the expiry date, unless stated otherwise below. Opened reagents must also be stored at +2°C to +8°C and protected from contamination.

- **Coated test strips:** Ready for use. Open the package with the test strips only when the strips have reached room temperature (+18°C to +25°C) to prevent condensation on the strips. After removal of the strips the package should be sealed tightly and stored at +2°C to +8°C.

- **Enzyme conjugate:** Ready for use. Mix thoroughly before using.

- **Universal buffer:** The universal buffer is supplied as a 10x concentrate. For the preparation of the working-strength universal buffer shake the bottle. The amount required should be removed from the bottle using a clean pipette tip and diluted 1:10 with deionised or distilled water. Due to the special membrane used for the present EUROLINE the working-strength universal buffer is used for the dilution of patient samples and the washing of the test strips. For the incubation of 1 test strip 5.0 ml buffer concentrate should be diluted with 45.0 ml water. The working-strength universal buffer should be used on the same working day.

- **Substrate solution:** Ready for use. Close bottle immediately after use, as the contents are sensitive to light  .

**Storage and stability:** The test kit must be stored at a temperature between +2°C and +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

**Waste disposal:** Patient samples and incubated test strips should be handled as infectious waste. Other reagents do not need to be collected separately, unless stated otherwise in official regulations.

**Warning:** Some of the reagents contain sodium azide in a non-declarable concentration. Avoid skin contact.

## Preparation and stability of the patient samples

**Sample material:** Human serum or EDTA, heparin or citrate plasma.

**Stability: Patient samples** to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

**Sample dilution:** The patient samples to be investigated are diluted 1:51 with the working-strength universal buffer using a clean pipette tip and mixed thoroughly by vortexing (for example, dilute 10 µl sample with 500 µl working-strength universal buffer). Sample pipettes are not suitable for mixing.

## Incubation

**Pre-treatment:** For every sample place one test strip 1 to 4 in separate incubation trays (Make sure that the surface of the test strips is not damaged!). Fill each of the channels with 1.0 ml working-strength universal buffer and incubate the test strips for **5 minutes**. Afterwards aspirate off all the liquid.

**Sample incubation:** Manual:
Fill each channel containing the test strip with **510 µl** of **1:51 diluted sample** using a clean pipette tip. Incubate for **60 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.

Automatic:
For the automated version with **1:51 diluted sample** the incubation volume must be increased to **1020 µl** (1.0 ml working-strength universal buffer plus 20 µl sample).

**Washing:** Aspirate off the liquid from each channel and wash for **3 x 5 minutes** with 1.0 ml working-strength universal buffer on a rocking shaker.

**Conjugate incubation:** Pipette 1.0 ml enzyme conjugate (alkaline phosphatase-labelled anti-human IgG) into each channel and incubate for **60 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.

**Washing:** Aspirate off the liquid from each channel. Wash as described above.

**Substrate incubation:** Pipette 1.0 ml substrate solution into each channel and incubate for **10 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.

**Stopping:** Aspirate off the liquid from each channel and wash each test strip **3 x 1 minute** with deionised or distilled water.

**Evaluate:** Place the test strip on the evaluation protocol, air dry and evaluate.

For automated incubation with the **EUROBlotMaster** select the program **Euro 09 FOOD**.

For automated incubation with the **EUROBlotOne** select the program **EURO 09 FOOD**.

# EUROLINE-FOOD Profile 216 (IgG)

## Incubation protocol

**Pre-treatment**
Place one test strip 1 to 4 in separate incubation channels and pre-incubate each strip with 1 ml working-strength universal buffer

5 min — Rocking shaker

**Manual sample incubation**
Aspirate off the fluid and pipette 510 µl of 1:51 prediluted sample into each channel

**Automatic sample incubation**
Aspirate off the fluid and pipette 1020 µl of 1:51 prediluted sample into each channel

60 min — Rocking shaker

**Washing**
Aspirate off, wash 3 x 5 min with 1.0 ml working-strength universal buffer each

**Conjugate incubation**
Aspirate off, pipette 1.0 ml enzyme conjugate into each incubation channel containing the test strip

60 min — Rocking shaker

**Washing**
Aspirate off, wash 3 x 5 min with 1.0 ml working-strength universal buffer each

**Substrate incubation**
Aspirate off, pipette 1.0 ml of substrate solution into the incubation channel containing the test strip

10 min — Rocking shaker

**Stopping**
Aspirate off, wash each strip 3 x 1 minute with deionised or distilled water

**Evaluation**
Fix the test strips to the evaluation protocol, air dry and evaluate

## Interpretation of results

**Handling:** After stopping the reaction using deionised or distilled water, place the incubated test strips onto the adhesive foil of the green work protocol (created beforehand in the EUROLineScan program) using a pair of tweezers. The position of the test strips can be corrected while they are wet. As soon as all test strips have been placed onto the protocol, they should be pressed hard using filter paper and left to air-dry. The drying process should take place without any direct light, in an environment as dark as possible. After they have dried, the test strips will be stuck to the adhesive foil. Incubated strips that are still moist show a background colouring that disappears when they are completely dry. Therefore the evaluation of the strips is only to take place after the strips have completely dried.

For **digital evaluation** follow the instructions in the EUROLineScan user manual. The code for entering the **test** into EUROLineScan is **FOOD strip 1** for test strip 1, **FOOD strip 2** for test strip 2, **FOOD strip 3** for test strip 3 and **FOOD strip 4** for test strip 4.

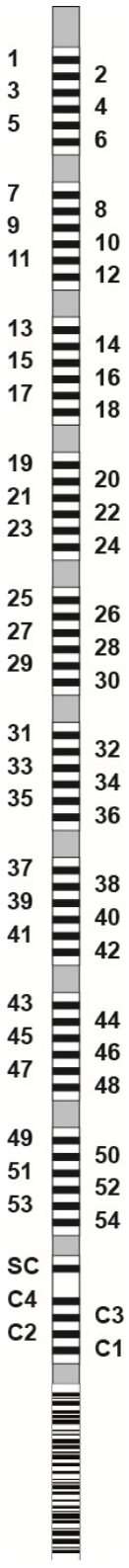
**Note:** EUROLineScan evaluates the test strips taking into account lot-specific specifications. These are transferred automatically to the software with the aid of barcodes on the EUROLINE. If the barcode is not readable, it is possible to enter it manually into EUROLineScan (see EUROLineScan instructions for use). The barcode number required can be found on the label of the folded card for the test strips.

**Caution:** A serum control band and 4 calibrators are located on the lower end of the strip. The correct performance of the incubation is confirmed after the automated evaluation of the staining intensity of the serum control band and the calibrators by means of the EUROLineScan program.

**Some samples might display a dark background staining of the membrane and white bands at the position of the antigens. These lighter bands should be interpreted as negative.**

# EUROLINE-FOOD

**Antigens:** The membrane strips are coated with the following foods and food additives.




| Position | EUROLINE-FOOD Profile 216 (IgG) | | | |
|---|---|---|---|---|
| | Strip 1 (3001-a) | Strip 2 (3001-b) | Strip 3 (3001-c) | Strip 4 (3001-d) |
| 1 | Barley flour | Cherry | Carob | Lime |
| 2 | Gluten | Grape, (white/ blue) | Rapeseed | Lychee |
| 3 | Oat flour | Kiwi | Duck meat | Mango |
| 4 | Rye flour | Lemon | Goat | Papaya |
| 5 | Spelt | Nectarine | Goose | Pomegranate |
| 6 | Wheat flour | Orange | Ostrich | Raspberry |
| 7 | Buckwheat flour | Pineapple | Quail | Rose hip |
| 8 | Flax seed | Strawberry | Rabbit | Cantaloupe |
| 9 | Corn | Watermelon | Roe deer | Raisin |
| 10 | Millet | Pear | Guinea fowl | Anise |
| 11 | Rice | Plum | Horse | Bay leaf |
| 12 | Beef | Grapefruit | Kefir | Chamomile |
| 13 | Chicken | Peach | Beta-Lactoglobulin | Caper |
| 14 | Lamb meat | Date fruit | Butter | Chive |
| 15 | Pork, cooked | Basil | Camembert | Clove |
| 16 | Turkey | Pepper, (black/ white) | Casein | Coriander |
| 17 | Cow´s milk | Cinnamon | Emmental cheese | Cumin |
| 18 | Egg yolk | Garlic | Cottage cheese | Dill |
| 19 | Egg white | Mustard seed | Mozzarella | Ginger |
| 20 | Goat´s cheese | Nutmeg | Processed cheese | Marjoram |
| 21 | Goat´s milk | Oregano | Curd cheese | Saffron |
| 22 | Sheep´s milk | Parsley | Bamboo shoots | Sage |
| 23 | Sheep´s cheese | Peppermint | Brussel sprouts | Cayenne pepper |
| 24 | Yogurt | Poppy seed | Cauliflower | Curry |
| 25 | Eggplant | Rosemary | Chard | Tarragon |
| 26 | Beetroot | Thyme | Chinese cabbage | Hops |
| 27 | Bell pepper | Vanilla | Fennel | Mint |
| 28 | Broccoli | Almond | Gourd | Brazil nut |
| 29 | Carrot | Cashew nut | Jerusalem artichoke | Macadamia nut |
| 30 | Celery | Cocoa bean | Kale | Pine nut |
| 31 | Chili | Hazelnut | Radish | Chestnut |
| 32 | Cucumber | Peanut | Savoy cabbage | Cola nut |
| 33 | Horseradish | Pistachio | Sweet potato | Carp |
| 34 | Leek | Sesame | Vine leave | Squid |
| 35 | Olive | Sunflower seed | Green cabbage | Eel |
| 36 | Onion | Walnut | Shallot | Gilthead seabream |
| 37 | Potato | Coconut | Liquorice root | Haddock |
| 38 | Red cabbage | Mushroom mixture 1* | Snow pea | Pike |
| 39 | Tomato | Mushroom mixture 2* | Broad bean | Turbot |
| 40 | Turnip | Crayfish | Chickpea | Herring |
| 41 | Zucchini | Salmon | Mung bean | Lobster |
| 42 | Artichoke | Tuna | Kidney bean | Mackerel |
| 43 | Asparagus | Clam | Chicory | Octopus |
| 44 | Spinach | Prawn | Iceberg lettuce | Oyster |
| 45 | String bean | Anchovy | Rocket | Sardine |
| 46 | Pea | Swordfish | Avocado | Ocean perch |
| 47 | Soya bean | Trout | Blackberry | Sea bass |
| 48 | Lentil | Sole | Blueberry | Caviar |
| 49 | White bean | Codfish | Cranberry | Crab |
| 50 | Lettuce | Brewer's yeast | Red currant | Agar-agar |
| 51 | Corn salad | Baker's yeast | Black currant | Aloe vera |
| 52 | Apple | Honey | Fig | Green tea |
| 53 | Apricot | Coffee | Gooseberry | Baking powder |
| 54 | Banana | Black tea | Honeydew melon | Safflower oil |
| SC | Serum control band | Serum control band | Serum control band | Serum control band |
| | | | | |
| C4 | Calibrator 4 | Calibrator 4 | Calibrator 4 | Calibrator 4 |
| C3 | Calibrator 3 | Calibrator 3 | Calibrator 3 | Calibrator 3 |
| C2 | Calibrator 2 | Calibrator 2 | Calibrator 2 | Calibrator 2 |
| C1 | Calibrator 1 | Calibrator 1 | Calibrator 1 | Calibrator 1 |

*Mushroom mixture 1: Oyster mushroom, White mushroom, Shiitake, Chanterelle
Mushroom mixture 2: Bay Boletus, Boletus

**Interpretation of results:**
When using EUROLineScan the intensity of the bands is measured by the software and converted into classes 0 to 4. The specific IgG titers are also given in U/ml*, which are calculated for each test strip in relation to the intensity of the calibrators.

The classes can be divided into the following concentrations:

| Class | Concentration [U/ml*] | Result | Interpretation |
|---|---|---|---|
| 0 | 0 < IgG ≤ 7.5 | Negative | No specific antibodies detectable |
| 1 | 7.5 < IgG ≤ 12.5 | Weak | Antibody titer in physiological range, no disease association |
| 2 | 12.5 < IgG ≤ 20 | Increased | Sensitisation possible |
| 3 | 20 < IgG ≤ 50 | Positive | Indicating sensitisation, food intolerance likely |
| 4 | > 50 | Strongly positive | Very high antibody titer, almost always associated with sensitisation and manifest intolerance |

*The EUROLINE-FOOD tests were calibrated using the WHO reference serum "1st IRP 67/86". The measurement values are given in units per milliliter [U/ml]. The limits given are reference values obtained in internal analysis.

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

## Test characteristics

**Measurement range:** The EUROLINE is a semiquantitative method. The measurement range is given in classes 0 to 4.

**Limitations of the determination:** The antibody determination indicates the patient's level of sensitisation to the different foods and food additives investigated. It does not, however, provide information on the connection between the height of the IgG antibody titer determined with the EUROLINE-FOOD and the occurrence or severity of food intolerance.

Furthermore, it cannot be excluded that epitopes become inaccessible for binding to the antibodies during coating of the strips, which can result in missing signals and thus in false negatives. It is also possible that antibody binding sites are only created or become free for binding during (industrial) processing or the digestion process. These cases can also cause false negative results.

**Cross-reactions:** If the antigenic structures are similar, e.g. in chemically similar substances or due to a close botanic relationship, cross-reactions between IgG antibodies of different foods and food additives may occur. The specific IgG antibodies that have developed in a patient also attach to identical epitopes of a homologous antigen.

**Interferences:** Haemolytic, lipaemic and icteric samples up to a concentration of 5 mg/ml for haemoglobin, of 20 mg/ml for triglycerides and of 0.4 mg/ml for bilirubin showed no effect on the analytical results of the EUROLINE-FOOD.

**Inter- and intra-assay variation:** The inter-assay variation was determined by multiple analyses of characterised samples over several days. The intra-assay variation was determined by multiple analyses of characteristic samples on one day. In every case, the intensity of the bands was within the specified range. The EUROLINE-FOOD displays an excellent inter-assay and intra-assay reproducibility.

## Clinical significance

Food intolerance (food hypersensitivity) is an immunological reaction to food or food additives. The immune response arises due to an increased permeability of the small intestine, which allows food components to leak through the intestinal wall and into the bloodstream. This permeability (so-called leaky gut syndrome) can be caused by diet-related hyperacidity of the gut flora, medication, infections, preservatives, alcohol/nicotine, stress and further factors.

The immune system identifies these antigenic structures as foreign and starts to produce specific antibodies of class IgG (sensitisation). This immune response, which typically occurs after a certain period of time (several hours to days after intake of the food), triggers an inflammatory reaction, which may become chronic. Chronic inflammation can manifest in the entire body, not only in the gastrointestinal tract. The most frequent symptoms are diarrhoea, constipation, bloating, nausea, upset stomach, irritable colon, migraine, asthma, joint disorders, lack of concentration, skin disorders and weight problems (over-/underweight). To relieve symptoms, an elimination diet (exclusion diet) for a set period of time is usually recommended. Those foods for which (high) IgG antibody concentrations were measured are excluded from the patient's diet. Improving the gut flora with therapeutic measures can also be taken into account to reduce or prevent the permeability of the intestinal wall to food antigens.

The association with different diseases has been described in many studies. It is often accompanied by high levels of IgG antibodies against various foods or food additives. This is the case in patients with headaches/migraine, arthritis, irritable colon (irritable bowel syndrome), inflammatory bowel diseases, asthma and a number of other diseases.

**Headaches and migraine:** A study from the 1930s already showed that 66% of 127 migraine patients were free of complaints after undertaking an elimination diet (Sheldon and Randolph, 1935). In other more recent studies these results were confirmed and it was shown that an elimination diet based on IgG levels significantly influences the frequency of migraine attacks (Alpay et al., 2010; Arroyave Hernández et al., 2007; Aydinlar et al., 2012; Mitchell et al., 2011).

**Arthritis:** Food intolerance has been known for more than 35 years to be a causative factor for developing arthritis. In a study with 22 patients with rheumatoid arthritis undertaking an elimination diet, 20 patients (91%) experienced an improvement of symptoms. 19 patients again suffered a deterioration of their health status when the reactive foods were reintroduced into their diet (Hicklin et al., 1980).

**Irritable colon:** In various studies with patients with irritable bowel syndrome the participants were treated with an elimination diet. A significant number of patients experienced an improvement of symptoms up to an at least subjective full recovery (Jones et al., 1982; Nanda et al., 1989). Atkinson and colleagues, and Drisko and colleagues performed studies with respect to IgG-based elimination diet in irritable-colon patients. Both groups showed that the symptoms could be reduced significantly (Atkinson et al., 2004; Drisko et al., 2006).

**Intestinal bowel diseases:** In a retrospective study Cai and colleagues (2014) investigated 112 patients with inflammatory bowel diseases [Crohn's disease (n = 79) and ulcerative colitis (n = 33)] and compared the data to those obtained in a healthy control group (n = 266). They found out that patients with inflammatory bowel diseases have a high prevalence of antibodies of class IgG against certain foods and that this can be used for establishing an elimination diet (Cai et al., 2014). Further studies yielded similar results (Lindberg et al., 1992; Bentz et al., 2010; Kawaguchi et al., 2014).

Increased levels of IgG antibodies against foods or food additives have also been observed in **asthma** (Calderon et al., 2010; Codina et al., 1997), **autism** (Lucarelli et al., 1995; Kidd, 2002) or **atopic dermatitis** (Shakib et al., 1977).

The internet-based study of Gaby (1998) gives an overview of the possible association of food intolerance with various diseases.

The diagnostic value of IgG (or IgG4) antibody detection for the diagnosis of food intolerance is being controversially discussed. Different studies with up to several thousand patient samples, however, have come to the conclusion that the determination of the antibody titer can be a useful tool for the identification of food intolerances and for targeted patient therapy (Atkinson et al., 2004; Zar et al., 2005; Bernardi et al., 2008; Volpi and Maccari 2009). Generally, it could be shown that elimination of those foods, against which strong antibody reactions were measured, helped to improve symptoms or to promote complete recovery in a statistically significant number of patients.

In a large internet-based study, Mullin and colleagues investigated the diagnostic benefit of IgG determination as a marker of food intolerance, and its clinical relevance. The authors concluded that IgG determination provides a clinically useful basis for establishing an elimination diet (Mullin et al., 2010).

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