EUROLINE Inhalation South East Asia (IgE)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
DP 3113-1601 E	Inhalation allergens	IgE	Test strips coated with allergens	16 x 01 (16)

Indication: The EUROLINE test kit enables the detection of specific IgE to support the diagnosis of sensitisations that may lead to allergy-associated symptoms, e.g. conjunctivitis, rhinitis or gastro-intestinal problems.

Principle of the test: The test kit contains test strips coated with 21 different allergens. The test strips are first moistened and then incubated with patient samples in the first reaction step. If samples contain specific antibodies of class IgE, they will bind to the allergens coated on the strip. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgE (enzyme conjugate) catalysing a colour reaction.

Contents of a test kit:

Description	Format	Symbol
1. **Test strips coated with the allergens:** ts19, t19, t223, u85, gs1, ds1, i6, u134, e1, e2, es172, e6, e71, e82, e84, ms1, ms4, m5, m12, m45, CCD	16 strips	STRIPS
2. **Enzyme conjugate** Alkaline phosphatase-labelled anti-human IgE (mouse), ready for use	1 x 30 ml	CONJUGATE
3. **Universal buffer** 10x concentrated	1 x 100 ml	BUFFER 10x
4. **Substrate solution** Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use	1 x 30 ml	SUBSTRATE
5. **Incubation tray,** volume-reduced (400 µl)	2 x 10 channels	TRAY
6. **Instruction booklet**	1 booklet	-

LOT Lot description — CE — Storage temperature

IVD In vitro diagnostic medical device — 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)
ZD 9895-0130 Incubation tray (volume 1 ml) with 30 channels (black, compatible with EUROBlotMaster and EUROBlotCamera System)
ZD 9898-0144 Incubation tray (volume 1 ml) with 44 channels (black, compatible with EUROBlotOne, EUROBlotMaster and EUROBlotCamera System)

Updates with respect to the previous version are marked in grey.

For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** you require green paper and adhesive 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: This test kit may only be used by trained personnel. Test strips and incubation trays are intended for single use. 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 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 2.0 ml buffer concentrate should be diluted with 18.0 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:
Version a: The patient samples to be investigated are used undiluted.
Version b: Dilute 175 µl patient sample with 250 µl working-strength universal buffer and mix thoroughly by vortexing. The final volume should be 425 µl.
Version c: Dilute 100 µl patient sample with 1.0 ml working-strength universal buffer and mix thoroughly by vortexing. The final volume should be 1.1 ml.
Sample pipettes are not suitable for mixing.

Incubation

Pretreat: Place the required amount of test strips in the incubation tray. 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: (1st step)

Manual:

Version a (time-optimised): Fill each channel of the volume-reduced incubation tray with **400 µl** of **undiluted sample** and incubate for **60 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.

Version b (volume-/time-optimised): Fill each channel of the volume-reduced incubation tray with **425 µl** of **diluted sample** (175 µl sample + 250 µl working-strength universal buffer) and incubate for **2 h** at room temperature (+18°C to +25°C) on a rocking shaker.

Version c (volume-optimised): Fill each channel with **1.1 ml of 1:11 diluted sample** and incubate **overnight (12 to 24 h)** on a rocking shaker at room temperature (+18°C to +25°C). The use of incubation trays with capacities of 1 ml and 400 µl is possible.
(Cover the incubation tray to prevent evaporation.)

Automatic:

Version b (volume-/time-optimised): The incubation volume must be increased to 510 µl (210 µl sample + 300 µl working-strength universal buffer).

Version c (volume-optimised): In version c with 1:11 diluted sample and incubation overnight, the incubation volume must be increased to 1.65 ml (150 µl sample + 1.5 ml working-strength universal buffer).

Caution: Version c is not suitable for volume-reduced trays, 1 ml trays should be used.

Washing:

Manual:
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.

Automatic:
In version c with 1:11 diluted sample and incubation overnight, a volume of 1800 µl must be used for the first wash step.

Conjugate incubation: (2nd step) Pipette 1.0 ml enzyme conjugate (alkaline phosphatase-conjugated anti-human IgE) into each channel.
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: (3rd step) Pipette 1.0 ml substrate solution into each channel.
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 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 **Euro11 Allerg EL60** (version a), **Euro08 Allerg 2h** (version b) or **Euro12 Allerg 16h** (version c).

For automated incubation with the **EUROBlotOne** select the program **EURO 11 Allergy EL60** (version a), **EURO 08 Allergy 2h** (version b) or **EURO 12 Allergy 16h** (version c).

EUROLINE Inhalation South East Asia (IgE)

Short protocol for manual test performance

Pre-treatment

- Insert test strip in an incubation channel
- Add 1.0 ml working-strength universal buffer by pipetting

5 min ↓ Rocking shaker

1. Sample incubation – three possible incubation options

- Remove liquid by aspiration or tilting
- Pipette samples into the tray channels, depending on the selected incubation option (ZD 9897, for option c also ZD 9895 or ZD 9898)

a) Time-optimised option	b) Time-optimised / volume-reduced option	c) Volume-reduced option
400 µl sample	250 µl working-strength universal buffer 175 µl sample	1 000 µl working-strength universal buffer 100 µl sample
400 µl total volume	425 µl total volume	1 100 µl total volume
60 min ↓ Rocking shaker	2 h ↓ Rocking shaker	12 – 24 h ↓ Rocking shaker

Washing

- Remove liquid by aspiration or tilting
- Wash test strips 3 × 5 min with 1.0 ml working-strength universal buffer each

2. Conjugate

- Remove liquid by aspiration or tilting
- Pipette 1.0 ml enzyme conjugate into the incubation channels containing the test strips

60 min ↓ Rocking shaker

Washing

- Remove liquid by aspiration or tilting
- Wash test strips 3 × 5 min with 1.0 ml working-strength universal buffer each

3. Substrate incubation

- Remove liquid by aspiration or tilting
- Pipette 1.0 ml substrate solution into the incubation channels containing the test strips

10 min ↓ Rocking shaker

Stopping

- Remove liquid by aspiration or tilting
- Wash test strips 3 × 1 min with deionised or distilled water

Evaluation

- Fix test strips on the protocol
- Air dry
- 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 **Inhalation South East Asia_V2**.

Staining of the indicator band confirms the correct use of all reagents included in the test kit. The test is considered to be valid if the evaluation of the indicator yields a result of at least EAST class 3. Values smaller than three are considered as invalid. In this case, the incubation should be repeated with fresh reagents.

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.

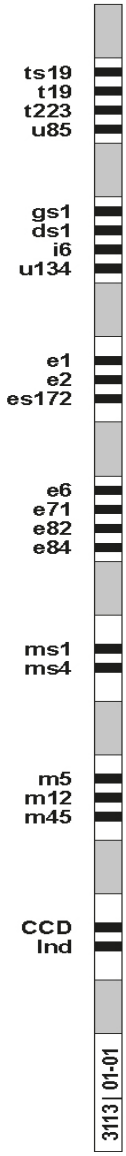
When using EUROLineScan the intensity of the bands is calculated in EAST classes of 0 to 6. EAST is the abbreviation for Enzyme-Allergo-Sorbent Test and is with respect to the concentration grades identical to the well-known RAST system (Radio-Allergo-Sorbent Test) used in allergy diagnostics.

The classes can be divided into the following concentrations:

Class	Concentration [kU/l]	Result
0	< 0.35	No specific antibodies detected.
1	0.35 ≤ sIgE < 0.7	Very low antibody titer, frequently no clinical symptoms where sensitisation is present.
2	0.7 ≤ sIgE < 3.5	Low antibody titer, existing sensitisation, frequently with clinical symptoms in the upper range of class.
3	3.5 ≤ sIgE < 17.5	Significant antibody titer, clinical symptoms usually present.
4	17.5 ≤ sIgE < 50.0	High antibody titer, almost always with clinical symptoms.
5	50.0 ≤ sIgE < 100.0	Very high antibody titer.
6	≥ 100.0	Very high antibody titer.

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

The test strips include the following allergens:



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Position	Allergen code	Allergen name
1	ts19	Tree mix 1 (melaleuca, acacia, eucalyptus and willow)
2	t19	Acacia
3	t223	Oil Palm
4	u85	Latex
5	gs1	Grass mix 1 (sweet vernal grass, bermuda; timothy grass and cultivated rye)
6	ds1	House dust mite mix 1 (Der. pteronyssinus, Der. farinae)
7	i6	Cockroach, German
8	u134	Kapok
9	e1	Cat
10	e2	Dog
11	es172	Cage bird mix 2 (budgerigar-, canary-, parrot-, lorebird- and finch feathers)
12	e6	Guinea pig
13	e71	Mouse
14	e82	Rabbit
15	e84	Hamster
16	ms1	Mould mix 1 (Penicillium notatum, Cladosporium herbarium, Aspergillus fumigatus and Alternaria alternata)
17	ms4	Mould mix 2 (Penicillium notatum, P. brevicompactum and P. roqueforti)
18	m5	Candida albicans
19	m12	Aureobasidium pullulans
20	m45	Curvularia spicifera
21	CCD	CCD marker
a	Ind	Indicator band

***The following labels may exist: SEAIN-01-01, 3113 | 01-01**

Test characteristics

Measurement range: The EUROLINE is a semiquantitative method. The measurement range is given in EAST system classes 0 to 6.

Conjugate specificity: The enzyme conjugate (alkaline phosphatase-labelled anti-human IgE (mouse)) does not show any measurable cross-reactivity for human IgA, IgD, IgG and IgM antibodies.

Cross-reactions: Due to the similar structure of the allergens, e.g. similarities in chemical substances or botanical relations, cross-reactions may occur. The specific IgE antibodies that have developed in a patient also attach to identical epitopes of homologous protein allergens.

Examples of cross-reactivity between airborne allergens and food allergens:

Inhalation allergens	***Associated food allergy***
Grass	Tomato, potato, carrot, celery, garlic, onion, wheat, rice, green pea, peanut, apple, peach, orange, melon, kiwi
Birch	Hazelnut, walnut, apple, pear, carrot, celery, potato, orange, kiwi
Mugwort	Celery, carrot, spices, mustard, hazelnut
Ragweed	Melon, cucumber, banana
English plantain	Melon
Latex	Avocado, potato, banana, tomato, walnut, kiwi

Interference: Haemolytic, lipaemic and icteric sera up to a concentration of 5 mg/ml haemoglobin, of 20 mg/ml triglycerides and 0.4 mg/ml bilirubin showed no effect on the analytical results of the present EUROLINE.

Inter- and intra-assay variation: The inter-assay variation was determined by multiple analyses of characteristic samples in several test runs over several days. The intra-assay variation was determined by multiple analyses of characteristic samples in one test run. In every case, the intensity of the bands was within the specified range. This EUROLINE displays excellent inter- and intra-assay reproducibility.

Sensitivity and specificity: The sensitivity of the EUROLINE with respect to the ImmunoCAP system is 90% for timothy grass (g6), 90% for birch (t3), 83% for Dermatophagoides pteronyssinus (d1), 84% for Dermatophagoides farinae (d2), 98% for cat (e1) and 82% for horse (e3).

The specificity of the EUROLINE with respect to the ImmunoCAP system is 100% for timothy grass (g6), 92% for birch (t3), 100% for Dermatophagoides pteronyssinus (d1), 86% for Dermatophagoides farinae (d2), 91% for cat (e1) and 100% for horse (e3).

Timothy grass (g6)		**EUROLINE**	
	n = 94	**pos.**	**neg.**
	pos.	64	7
ImmunoCAP	**neg.**	0	23

Birch (t3)		**EUROLINE**	
	n = 97	**pos.**	**neg.**
	pos.	55	6
ImmunoCAP	**neg.**	3	33

Dermatoph. pt. (d1)		**EUROLINE**	
	n = 44	**pos.**	**neg.**
	pos.	25	5
ImmunoCAP	**neg.**	0	14

Dermatoph. far. (d2)		**EUROLINE**	
	n = 45	**pos.**	**neg.**
	pos.	26	5
ImmunoCAP	**neg.**	2	12

Cat (e1)		**EUROLINE**	
	n = 74	**pos.**	**neg.**
	pos.	50	1
ImmunoCAP	**neg.**	2	21

Horse (e3)		**EUROLINE**	
	n = 34	**pos.**	**neg.**
	pos.	9	2
ImmunoCAP	**neg.**	0	23

Limitations of in vitro allergy diagnostics

Accurate performance of the assays according to the test instruction will lead to reliable and reproducible results. In any case, the final diagnosis should not be solely based on one type of analysis. A well-founded anamnesis and further laboratory findings should always be taken into account. A skin test as well as provocation test (if possible) is mandatory to receive the entire information needed for an optimal decision regarding the specific immunotherapy that should be applied. The clinical picture is not always in line with in vitro test results.

Negative in vitro results may occur e.g. when:

- symptoms are not IgE-mediated,
- samples were taken before the organism was able to produce antibodies against the antigen,
- IgE concentrations reached a minimum a long time after sensitisation.

Positive results with specific IgE in vitro tests do not necessarily have to correlate with clinical manifestations.

Many IgE antibodies can cross-react with various allergens or redundant carbohydrate structures.
Especially food allergens frequently show a negative result in vitro although clinical symptoms may be present. This phenomenon can be explained through the effect of maturing, industrial processing, cooking, or frying of the allergen. Furthermore, the allergic reaction can be induced by a metabolite of the allergen resulting from the digestive process, which cannot exactly be recapitulated by in vitro diagnostics. Allergens bind to various degrees to the solid phase, which can influence the test results.
For the determination of specific IgE antibodies a variety of test systems is available. Due to the variability of the source material used for the production of allergen extracts and the manufacturing process itself the quality of the extracts used for allergy diagnostics varies significantly. Therefore the results of different test systems cannot easily be compared to each other due to the lack of international standards for both the allergens and the antibodies used in these assays. Thus, a slight deviation between different test systems cannot be ruled out.
In general, identical results for different patients do not necessarily mean identical clinical manifestations.

Clinical significance

The term “allergy” was defined in 1906 by the Austrian paediatrician Clemens von Pirquet to mean the body’s increased ability to react to a foreign substance. Today “allergy” means an oversensitivity to foreign substances which are normally harmless. Alongside any genetic predisposition, numerous non-genetic factors also play a role, such as exposure to the allergen, nutritional condition, existing chronic diseases and acute viral infections. Atopy is a hereditary disposition to developing allergic reactions such as allergic asthma, rhinitis (hay fever) or dermatitis (including atopic eczema).

The most frequently occurring allergy is a type I hypersensitivity reaction, in which specific IgE antibodies are formed. The symptoms (rash, oedema or itching) generally occur shortly after contact with the allergen. These allergies are therefore also termed immediate-type reactions. Allergens are acquired either through the air and mucous membranes of the body (inhalation allergies) or by ingestion (food allergies).

More than 15% of the population in industrial countries suffer from an immediate-type allergy. Typical allergic reactions are rhinitis, conjunctivitis and allergic asthma. A worldwide increase in allergic rhinitis has been observed, with a prevalence of 4% to more than 40% in various regions. Inhalation allergies can be triggered by seasonal allergens (pollen from trees, grasses and weeds) or all-year-round indoor allergens (house dust mites, domestic animals, mould spores). The allergic symptoms intensify with every further exposure to the allergen. If a systemic allergic reaction occurs, serious, even life-threatening reactions can result (anaphylactic shock).

A food allergy is an IgE-mediated reaction which leads to symptoms within hours of having ingested the food. The most common foods causing allergic reactions are peanuts, soy, wheat, shellfish, fish, milk, eggs and tree nuts. Possible symptoms are burning or itching in the oral cavity, nausea, gastrointestinal spasms, diarrhoea and skin rashes. Severe reactions can also lead to asthma attacks, breathlessness, increased heart rate and to panic attacks and confusion. In rare cases anaphylaxis can occur (e.g. after consumption of peanuts, nuts or fish).

However, allergic reactions to foods of plant origin can also be caused by cross-reacting IgE antibodies. These reactions, termed cross-allergies, are based on the structural similarity of proteins which are present in both the food as well as in the corresponding inhalation allergens of plant origin. For example, patients with a birch pollen allergy can also develop allergic reactions to apple, celery, hazelnut, potato or kiwi.

Immunotherapy or hyposensitisation (desensitisation) established as a treatment for type I allergy does not provoke a change in IgE levels, although a significant reduction of the symptoms can be achieved. A definite response of a patient to immunotherapy normally manifests as an increase in the allergen-specific IgG antibody concentration in the course of treatment. However, this does not always correlate with a remission in symptoms.

Many allergens are glycoproteins and contain oligosaccharide side chains which are bound to the protein framework of the allergens. Some patients develop specific antibodies against these carbohydrate structures. The abbreviation CCD stands for cross-reactive carbohydrate determinant. CCDs are present in many plant and animal allergens. Due to their significant similarity in structure, CCDs are known to cause a strong cross-reactivity. Although the importance of specific IgE antibodies against CCDs has not yet been fully understood, they are considered to be irrelevant for diagnosis in most cases and as such complicate the interpretation of positive in vitro diagnostic results. For this reason, the determination of specific IgE antibodies against CCDs may provide useful additional information, especially when positive IgE results disagree with the clinical picture, and can serve as an interpretation aid in the evaluation of overall test results.

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