This package insert must be read carefully prior to use of this product.

EN

Dermatophyte Test Strip

Diafactory Tinea Unguium

For 10 tests

[General Precautions]

1. IVD Diafactory Tinea Unguium (this kit) is only intended for *in vitro* diagnostic use, and must not be used for any other purposes.
2. The user should evaluate the result of this assay comprehensively in conjunction with other test results and the clinical symptoms.
3. This kit should only be used as directed. The reliability of values cannot be guaranteed if this kit is used for other purposes or if tests are conducted by other methods than stated in the manual.

[Description (Kit Components)]

	Components	Ingredients
1	Test Strips	Anti-Dermatophyte Antibody Anti-Dermatophyte Antibody with Gold Colloid
2	Extraction Buffer	Buffer etc.

Accessories: Test tubes, stir rods

[Intended Use]

Detection of Dermatophyte-derived antigens in nails (in support of a diagnosis of tinea unguium)

[Principle of the Test]

Diafactory Tinea Unguium is a lateral flow immunoassay intended to detect Dermatophyte-derived antigens in nails using anti-Dermatophyte antibody that has been immobilized on a nitrocellulose membrane.
The test strip used in this kit is composed of a sample pad, a reagent pad, a test paper and an absorbent pad (Figure 1). The reagent pad contains anti-Dermatophyte antibody with gold colloid in the dry state, and the test paper contains anti-Dermatophyte antibody in the dry state affixed on the test line zone and the dye in the dry state affixed on the control line zone. This dye is a colorless dye at a pH of 3 that turns pink at a pH of approximately 4 or higher, and allows the user to confirm that a specimen has correctly passed through the test line zone.

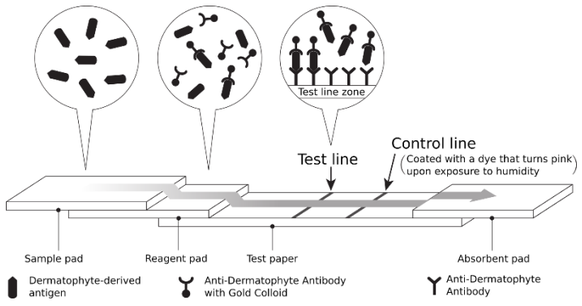
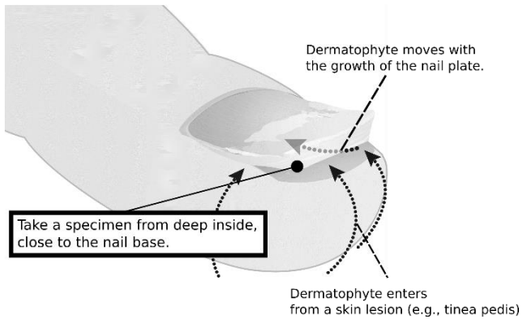


Figure 1. Principle of the Test

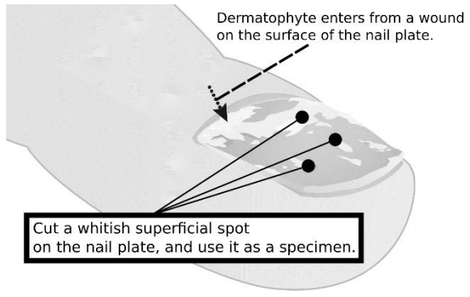
A sample that has infiltrated the sample pad (hereinafter called, "the extracted sample") moves to the reagent pad, on which a Dermatophyte-derived antigen in the extracted sample binds to an anti-Dermatophyte antibody with gold colloid to form an immune complex. While proceeding through the test paper, the immune complex is captured by the anti-Dermatophyte antibody affixed on the test line zone, resulting in the appearance of a purple line of gold colloid (in case it is positive). If the specimen does not contain Dermatophyte-derived antigen, no immune complexes are formed and the extracted sample containing unbound anti-Dermatophyte antibody with gold colloid passes over the test line zone without producing a visible band on the test line zone. The extracted sample containing unused anti-Dermatophyte antibody with gold colloid, whether it is Dermatophyte-derived antigen positive or negative, passes through the test line zone and reaches the control line zone, where the extracted sample reacts with immobilized dye, resulting in the appearance of a pink band.

[Procedural Precautions]

1. Precautions regarding specimens
   (1) This kit is intended for the detection of Dermatophyte-derived antigen in nails. Scales, scalp specimens, hair or other specimens cannot be used.
   (2) Take a specimen of 1 mg or more according to the guidelines for diagnosis and treatment of cutaneous fungal infection.[1,2,3] An inappropriate procedure for specimen collection or an insufficient amount of specimen taken may lead to false negative results or an incorrect judgment.
2. Specimen Collection and Preparation
   (1) Preparation for Specimen Collection
   According to the guidelines for diagnosis and treatment of cutaneous fungal infection,[1,2,3] take a specimen and place it in a test tube from the kit. Use clean nipper-type nail clippers or surgical scissors when taking a specimen.
   (2) Specimen collection must be performed by a professional who is qualified by appropriate education, training and/or experience according to the guidelines for diagnosis and treatment of cutaneous fungal infection. The procedure for specimen collection stated in the guidelines is partially shown below.
      1) Distal and Lateral Subungual Onychomycosis
      Remove the area of onycholysis or the tip of the nail, and take a specimen from the deep portion of the nail, as close to the nail bed as possible. If a specimen cannot be taken from the deep portion of the nail, take a specimen from the surface of the skin (actually the nail bed), where onycholysis is present.



      2) Superficial White Onychomycosis
      Remove a whitish superficial spot on the nail plate using nipper-type nail clippers or surgical scissors, and use it as a specimen.



   (3) It is desirable that the specimen amount should be 1 mg or more.
3. Influence of Drugs
   The influence of oral antifungals[1] (terbinafine, itraconazole) that are commonly used for the treatment of tinea unguium on this assay were evaluated. When the negative control specimen, the positive control specimen and the weakly positive specimen which was prepared by diluting the positive control specimen with the negative control specimen was mixed with the antifungals and subjected to this kit, no influences of these drugs were observed. The concentration of each drug added was approximately 100 times the MIC (minimum inhibitory concentration).

Table 1. Influence of Antifungals coexisting in Specimens

Antifungal	Concentration (μg/mL)	Influence
Terbinafine	0.5	Not observed
Itraconazole	100	Not observed

4. Other Precautions
   (1) Once the aluminum pouch of a test strip is opened, the test should be conducted immediately.
   (2) Do not bend or fold the test strip.
   (3) Do not touch or damage the sample pad zone of the test strip.
   (4) Put the set volume of extraction buffer in a test tube.
   (5) Each test strip, extraction buffer, test tube and stir rod in the kit can only be used once. Do not re-use these.
   (6) Read the result within 30 minutes. The result may be judged to be positive if colored bands are found both on the test line zone and the control line zone after at least 5 minutes have elapsed. Similarly, the result may be judged to be negative if no visible band appears on the test line zone and a band appears on the control line zone after at least 5 minutes have elapsed.

[Assay Procedure]

1. Assay Procedure
   The following procedure should be performed at room temperature (1 to 30ºC).
   (1) Prepare the required quantities of test strips, stir rods and extraction buffer.
   (2) Add 0.25 to 0.5 mL of the extraction buffer to the test tube (Figure 2). Put the specimen in the test tube and stir at least 20 times with a stir rod while pushing the specimen down. After stirring, stand the test tube in a test tube rack for at least 1 minute.

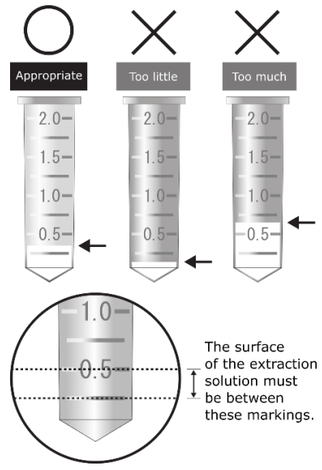


Figure 2

   (3) Cut the slit of the aluminum pouch and remove a test strip. Hold the test strip by the handle only. Do not touch the sample pad (Figure 3).
   (4) Stand the test strip in the test tube with the sample pad down. Confirm that the sample pad has reached the bottom of the test tube.
   (5) Let the test strip stand for at least 5 minutes and determine the result (positive, negative or invalid) by visually checking the presence or absence of colored bands in the control line zone and the test line zone, within 30 minutes after standing the test strip in the test tube.

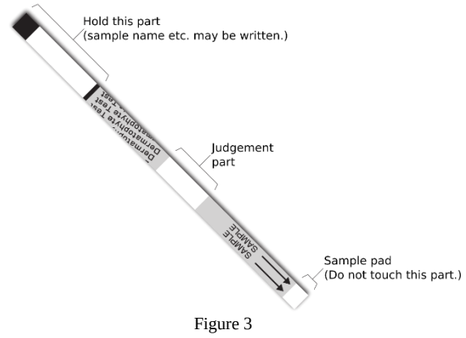


Figure 3

[Interpretation of Results]

Interpretation

   (1) If a pink band appears in the control line zone and a purple band appears in the test line zone, it indicates a Dermatophyte-derived antigen positive result. If a pink band appears in the control line zone and no visible band appears in the test line zone, it indicates a negative result.
   (2) If no pink band appears in the control line zone after 5 to 30 minutes, the test is invalid.
   (3) If a band appears in the test line zone after 30 minutes or longer, it indicates a negative result.

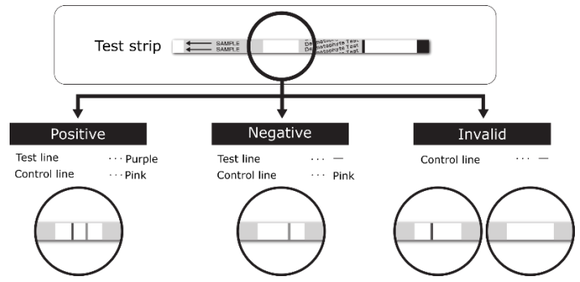


Figure 4

Precautions for Interpretation

1. If the amount of Dermatophyte in the nail specimen is small, the result may become negative. The user should comprehensively evaluate the result of this assay in conjunction with other test results and clinical symptoms.
2. This kit is cross-reactive with other fungi than Dermatophyte, such as *Aspergillus* and *Penicillium*. These fungi may be present in the soil or other environments and infect the skin of immune-compromised patients. Professionals should be careful when making a diagnosis.

[Clinical Significance]

Diafactory Tinea Unguium, unlike direct microscopy, does not require special skills to determine whether Dermatophyte is present or absent,[4] and this kit, unlike PCR, does not require special equipment. Diafactory Tinea Unguium, which is easy to use and provides quick results, is an effective assay for the rapid diagnosis of tinea unguium.

[Performance]

1. Performance
   (1) Sensitivity and Accuracy
   When a negative control specimen was tested, this kit provided a negative result.
   When a weakly positive specimen and a positive control specimen were tested, this kit provided positive results.
   (2) Within-run reproducibility
   When a negative control specimen was tested 4 times, the kit provided a negative result every time.
   When a weakly positive specimen and a positive control specimen, respectively, were tested 4 times, the kit provided a positive result every time.
   (3) Minimum Detectable Sensitivity
   Trichophyton rubrum (NBRC 9185), 0.5 μg dry weight/mL
   (4) Reference Standard for Calibration
   Dry cells of *Trichophyton rubrum* (NBRC 9185)
   (5) Cross-reactivity
   Autoclaved dry cells of various other fungi than Dermatophyte were added to the extraction buffer at a concentration of 300 μg/mL to evaluate the influence of each fungus on the assay. In addition, colonies of various bacteria grown on agar plates were added to the extraction buffer to evaluate the influence of each bacterium on the assay. This kit was not reactive with the tested fungi (non Dermatophyte) shown below.
   Aspergillus nidulans, Penicillium citrinum, Scopulariopsis brevicaulis, Alternaria alternate, Pseudallescheria boydii, Scedosporium apiospermum, Prototheca wickerhamii, Schizophyllum commune (1 nucleus), Schizophyllum commune (2nucleii), Absidia corymbifera, Basidiobolus ranarum, Cunninghamella bertholletiae, Mortierella isabellina, Mucor circinelloides, M. racemosus, Rhizomucor pusillus, Rhizopus microsporus var. rhizopodiformis, R. oryzae, R. stolonifer var. reflexus, Syncephalastrum racemosum,

Zygorhynchus exponens, Candida albicans, C. dubliniensis, C. tolopicalis, C. parapsilosis, C. guilliermondii, C. glabrata, C. krusei, Geotrichum candidium, Trichosporon asahii, Cryptcoccus neoformans serotype A, C. neoformans serotypeB, C. neoformans serotype C, C. neoformans serotype D, C. neoformans serotype AD, Sporothirix schenckii, Fonsecaea pedrosoi, Exophiala. jeanselmei, Phialophora verrucosa, P. richardsiae, Rhinocladiella atrovirens, Cladophialophora bantiana, Malbranchea albolutea, M. aurantiaca, M. chrysosporioidea hrysosporioidea, M. cinnamomea, M. dendritica, M. filamentosa, M. flava, M. flocciformis, M. fulva, M. graminicola, M. gypsea, M. multicolor, M. pulchella, Malassezia furfur, Gymnoascoideus petalosporus, Auxarthron reticulatum, Gymnoascus intermedius, G. petalosporus, G. reessii, G. udagawae, Emmonsia parva var. crescens, E. parva var. parva, Phanerochaete chrysosporium, Apinisia queenslandica, Arthroderma multifidum, Uncinocarpus reesii, Chrysosporium carmichaelii, C. indicum, C. keratinophilum, C. pseudomerdarium

The kit was reactive with the fungi (non Dermatophyte) shown below.

Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Neosartorya fischeri, Paecilomyces lilacinus, Penicillium griseofulvum, Veronaea botryosa, Fusarium solani, Exophiala dermatitidis (M-Y form), E. dermatitidis (G form), E. spinifera, Hortaea werneckii, Malbranchea circinata, M. flavorosea

The kit was not reactive with the bacterium shown below.

Escherichia coli, Bacillus subtilis, Staphylococcus aureus, S. faecalis

(6) Reaction with Dermatophyte

Autoclaved dry cells of Dermatophyte were added to the extraction buffer at a concentration of 300 μg/mL to evaluate the reactivity of the assay. The kit was reactive with the Dermatophyte shown below.

Trichophyton mentagrophytes, T. rubrum, T. tonsurans, T. violaceum, T. verrucosum, Microsporum gypseum, M.canis, Epidermophyton floccosum

2. Results of Clinical Performance Study[5]

In 222 patients (at 11 centers) suspected of having tinea unguium on visual inspection, a specimen was collected from a foot or hand nail according to the guidelines for diagnosis and treatment of cutaneous fungal infection. The specimen was crushed into 3 pieces and subjected to measurement with this kit, direct microscopy and PCR (only specimens for which the results of this kit and direct microscopy were inconsistent), respectively. Specimen collection, direct microscopy, this kit and PCR were performed by different persons under blinded conditions.

(1) Comparison between the results of Diafactory Tinea Unguium and PCR incorporating direct microscopy

Analyses were performed on 222 patients. In 5 patients in whom the results of this kit and direct microscopy were inconsistent and PCR could not be performed because the amount of specimen was insufficient, the result of direct microscopy was used.

Table 2. Comparison between the Results of Diafactory Tinea Unguium and PCR incorporating Direct Microscopy

		PCR incorporating direct microscopy		
		Positive	Negative	Total
Diafactory Tinea Unguium	Positive	196	5	201
	Negative	6	15	21
	Total	202	20	222

Sensitivity, 97.0%
Specificity, 75.0%
Accuracy, 95.0%
Negative predictive value, 71.4%
Positive predictive value, 97.5%

(2) Comparison between the results of Diafactory Tinea Unguium and the dermatologist's final diagnosis (based on the results of direct microscopy, PCR, clinical manifestation, specimen collection site, etc.)

Analyses were performed on 217 patients, excluding 5 patients in whom PCR could not be performed because the amount of specimen was insufficient and a final diagnosis could not be made.

Table 3. Comparison between the Results of Diafactory Tinea Unguium and the Final Diagnosis

		Final diagnosis		
		Tinea unguium	Not tinea unguium	Total
Diafactory Tinea Unguium	Positive	196	2	198
	Negative	4	15	19
	Total	200	17	217

Sensitivity, 98.0%
Specificity, 88.2%
Accuracy, 97.2%
Negative predictive value, 78.9%
Positive predictive value, 99.0%

[Precautions for Use and Handling]

1. Precautions for Handling (to Prevent Danger)

(1) If the extraction buffer comes in contact with the eyes, mouth, or skin, rinse thoroughly with running water as first aid, and seek medical treatment if necessary.

(2) When handling specimens and this kit, wear a mask, gloves and other protective apparel. Wash hands thoroughly after testing.

(3) All specimens used for the test should be handled as if potentially infectious. Used test strips, extracted samples, test tubes and stir rods should be handled in the same manner as specimens.

(4) To prevent infections from spilled specimens or solutions containing specimens, wipe the spilled and contaminated area thoroughly with disinfectant such as sodium hypochlorite solution.

2. Precautions for Use

(1) The reagents in this kit can be used only for the detection of Dermatophyte-derived antigens in nails.

(2) Use clean instruments when taking specimens.

(3) Do not use the kit beyond the expiration date.

(4) Each test strip, extraction buffer, test tube and stir rod in the kit can only be used once. Do not re-use them.

(5) This kit should be stored at 2 to 30ºC. Avoid freezing and exposure to direct sunlight.

(6) Do not combine reagents of different lots.

3. Precautions on Disposal

(1) Before disposal, used test strips and containers must be autoclaved at 121ºC for 20 minutes or soaked in a sodium hypochlorite solution for longer than an hour, as if potentially infectious.

(2) Used containers etc. must either be incinerated or disposed of as medical or industrial waste according to the applicable waste disposal regulations.

[Storage and Shelf Life]

Storage temperature, 2 to 30°C (Do not freeze)

Shelf life, 36 months from the date of manufacture (The expiration date is printed on the outer package.)

[Package Contents]

REF DE001 ------- 10 tests/kit

[References]

1. Ameen M, Lear JT, Madan V, Mohd Mustapa MF, Richardson M. British Association of Dermatologists' guidelines for the management of onychomycosis 2014. Br J Dermatol 2014; 171: 937–958.
2. Guidelines of care for superficial mycotic infections of the skin: onychomycosis. Guidelines/Outcomes Committee. American Academy of Dermatology. J Am Acad Dermatol 1996; 34: 116-121.
3. British Association of Dermatologists: Guidelines for treatment of onychomycosis , Roberts DT., Taylor WD., Boyle J., Br. J. Dermatol., 148, 402-410, 2003
4. Screening for tinea unguium by Dermatophyte Test Strip, Y. Tsunemi, et al., Br. J. Dermatol. , 170, 328-331, 2014
5. Clinical study of Dermatophyte Test Strip, an immunochromatographic method, to detect tinea unguium dermatophytes, Y. Tsunemi, M. Hiruma, J Dermatol., 43, 1417, 2016

[Symbol Legend]

	Consult instructions for use
IVD	*In vitro* diagnostic medical device
	Do not re-use
	Caution, consult accompanying documents
	Keep away from sunlight
30 °C 2 °C	Temperature limitation
	Use-by
Σ	Contains sufficient for <n> tests
REF	Catalogue number
LOT	Batch code
	Manufacturer
EC REP	Authorized representative in the European Community

[Manufacturer]

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CE

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