HERPES (1, 2) FA Reagent "SEIKEN"

Herpes simplex virus (HSV) is classified into two types, type 1 (HSV-1), called labial herpes virus, and type 2 (HSV-2), also known as genital herpes virus. To distinguish between the two in the diagnosis of herpetic disease, it is thus important to identify the HSV type in specimens. In recent years, the use of antibodies with high specificity, especially monoclonal antibodies, has been useful in allowing both direct detection and type identification of HSV antigens.
This reagent contains monoclonal antibodies that are labeled with fluorescein isothiocyanate (FITC), specific for HSV-1 and HSV-2 and can be used for the direct detection and type-identification of HSV in specimens.

CHARACTERISTICS

The FITC-labeled monoclonal antibodies employed in this kit can directly detect and identify antigen type rapidly and specifically.

CONTENTS

1. **Anti-HSV-1 monoclonal antibody labeled with FITC** **1 ml x 1 vial**
   Lyophilized anti-HSV-1 mouse monoclonal antibody labeled with FITC. Contains bovine serum albumin as a stabilizer.
2. **Anti-HSV-2 monoclonal antibody labeled with FITC** **1 ml x 1 vial**
   Lyophilized anti-HSV-2 mouse monoclonal antibody labeled with FITC. Contains bovine serum albumin as a stabilizer.
2. **Diluent** **3 ml x 1 vial**
   Purified distilled water containing 0.01w/v% Evans blue and 0.1w/v% sodium azide.
3. **Inclusion Liquid** **3 ml x 1 vial**
   Phosphate buffer containing 1v/v% glycerin

INTENDED USE

To detect and type-identify HSV-1 and HSV-2 in patient samples from infections of the lips, urinary organs or genitalia.

PRINCIPLE

The reaction between type-specific anti-HSV monoclonal antibodies (mouse) labeled with FITC and herpes virus is observed by fluorescent microscopy.

PROCEDURE

1. **Instruments and equipment**
   Epi-illumination transmission fluorescent microscope
   Non-fluorescent slide
   Micropipette
2. **Preparation of specimen**
   Using a swab collect cells from an affected area of the lips, urinary organs or genitalia. Spread the collected cells evenly on a non-fluorescent slide and dry thoroughly with cool air. Fix the cells by dipping in cold acetone for 5 to 10 minutes.

3.Preparation of reagent

Prepare antibody solutions by adding 1 ml of diluent to the bottle containing FITC labeled anti-HSV-1 monoclonal antibody and to the other containing FITC labeled anti-HSV-2 monoclonal antibody After reconstitution, keep the solutions at 2 to 10℃ for 15 minutes before use. The reconstituted antibody solutions should be stored at 2 to 10 ℃ and used within 3 months.

4. Measurement

1) After applying 30 μ l of each antibody solution to a spread slide, place the slide in a moisture box for 15 minutes at 37℃. Avoid drying the antibody solutions on the slide during the incubation otherwise non-specific reaction may occur.
2) Using a wash bottle, wash off excess reagent from the slide with distilled water but avoid directly squirting the area of the slide where the specimen is spread.
3) Dry the slide with cool air and enclose in inclusion liquid, and then observe it under a microscope (200 - 500 x). If the observation will not be done immediately, store the slide at 2 ~10℃ protected from light and observe within 24 hours.

NOTE

1. Do not mix the two antibody solutions since monoclonal antibodies are highly specific.
2. Before performing the inclusion procedure, confirm that the sample is thoroughly dried; otherwise the background will appear hazy upon microscopy.

INTERPRETATION OF RESULTS

Cells infected with HSV will emit a specific green fluorescence which is easily seen against the red background of non-infected cells. If the area of the slide exposed to the anti-HSV-1 monoclonal antibody emits a specific fluorescence, infection with HSV-1 is indicated; likewise, if the area exposed to anti-HSV-2 monoclonal antibody emits a specific fluorescence, infection with HSV-2 is indicated.

As a positive or negative result obtained through the use of a single reagent can not exclude the possibility of infection by the other, it is recommended that both reagents be used when performing typing and identification. If fluorescence specific for both monoclonal antibodies is observed, HSV-1 and HSV-2 coinfection is suspected.

However, final judgment should be made only after giving an overall consideration to the patient's clinical history and symptoms.

PERFORMANCE

1. Sensitivity test

When the antibody solutions containing either the FITC-labeled anti-HSV-1 monoclonal antibody or anti-HSV-2 monoclonal antibody were serially diluted four-fold and used in the slide test, specific fluorescence was clearly observed.

2. Specificity test

1) When control slides, one spread with HSV-1 infected cells and the other with HSV-2 infected cells, were used as samples, specific fluorescence was seen only when the corresponding FITC-labeled monoclonal antibody was used.
2) When cells infected with a virus (chickenpox/cingule virus or cytomegalovirus) of the same genus as herpes virus, neither the anti-HSV-1 monoclonal antibody nor the anti-HSV-2 monoclonal antibody showed specific fluorescence.

3. Reproducibility test

When control slides separately spread with HSV-1 infected cells and HSV-2 infected cells were tested as samples simultaneously five times, anti-HSV-1 monoclonal antibody showed specific fluorescence only with cells infected with control HSV-1, and, likewise, anti-HSV-2 monoclonal antibody labeled with FITC showed specific fluorescence only with cells infected with control HSV-2.

CORRELATION

The correlation between HERPES (1, 2) Reagent FA "SEIKEN" and another company's reagent, in a comparative study where 101 samples obtained from lips, urinary organs and genitalia infection were tested, is shown in the following data;

HSV-1		Company A	
		+ -	—
HERPES (1, 2) Reagent FA "SEIKEN"	+	26	0
	—	0	75

HSV-1		Company A	
		+	—
HERPES (1, 2) Reagent FA "SEIKEN"	+	12	0
	—	0	89

+ : Specific fluorescence was observed
— : Specific fluorescence was not observed

PRECAUTION

1. Do not expose FITC-labeled antibodies reagent to strong light.
2. To prevent contamination of specimens, pipette tips should be changed for each specimen.
3. Trypsinizing specimens may give poor results.
4. When contacting the reagents with skin or cloths, wash off immediately.
5. All specimens and equipment used in the test should be sterilized by one of the following methods after use:
   1) Soaking in 2% glutaraldehyde for one hour or longer.
   2) Soaking in 0.5% sodium hypochlorite solution (effective chloride: approx. 5000 ppm) for one hour or longer.
   3) Autoclaving at 121℃ for 20 minutes or longer.
6. The reagents contains sodium azide as a preservative. As sodium azide may react with lead or copper piping to form highly explosive metal azide, discard of reagents by flushing with copious amounts of water.

STORAGE AND SHELF LIFE

Storage: 2～10℃ protected from the light.
Shelf life: Up to expiry date on the label

PACKAGE

HERPES (1, 2) FA Reagent "SEIKEN" for 36 specimens (Product code: 421801)