For Research Use Only

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SERODIA®·HTLV-I

Passive Particle-Agglutination Test
for Detection of Antibodies to HTLV-I

This kit is reagent for research and cannot be used for diagnostics.
Always read the instruction manual prior to use, and use according to the instructions.

PRINCIPLE AND ADVANTAGES:

The reagent is prepared from gelatin particles carrier sensitized with HTLV-I* (Human T-Lymphotropic Virus Type 1) on the principle that these sensitized particles are agglutinated by anti-HTLV-I antibody in Human serum or plasma. HTLV-I is prepared by disrupting purified HTLV-I with detergent.

*Note 1) This is prepared by concentrating the culture fluid of a virus-producing cell line, sub-jecting it to sucrose-gradient centrifugation, collecting the virus fraction corresponding to a density of about 1.16 g/cm^3.

SERODIA·HTLV-I has the following advantages:

1. The test procedure is extremely simple as a microtiter technique and is particularly suitable for mass-screening of test samples.
2. The test is time-saving and results are readable by the naked eye after about 2 hours.
3. SERODIA·HTLV-I involves the use of a newly developed artificial carrier Fuji particle that does not Show the nonspecific agglutination usually observed with red cell carriers.

KIT COMPONENTS

SERODIA·HTLV-I consists of the following reagents and accessories.

Maximum Assays	REAGENTS				
	Reconstituting Solution (Liquid)	Sample Diluent (Liquid)	Sensitized Particles (Lyophilized)	Unsensitized Particles (Lyophilized)	Positive Control (Liquid)
Screening: 100 (20 × 5)	10 mL × 1 vial	16 mL × 1 vial	0.6 mL × 5 vials*	1.0 mL × 5 vials*	0.5 mL × 1 vial
Screening: 220 (55 × 4)	20 mL × 1 vial	35 mL × 1 vial	1.5 mL × 4 vials*	2.0 mL × 4 vials*	0.5 mL × 1 vial
Screening: 550 (110 × 5)	20 mL × 2 vials	35 mL × 2 vials	3.0 mL × 5 vials*	3.5 mL × 5 vials*	0.5 mL × 1 vial

*Note 2) After reconstitution

A: Reconstituting Solution (Liquid): Use for reconstitution of Sensitized Particles, Unsensitized Particles. This reagent contains 0.10% (w/v) of sodium azide as preservative.

B: Sample Diluent (Liquid): Use for dilution of specimens. This reagent contains 0.10% (w/v) of sodium azide as preservative.

C: Sensitized Particles (Lyophilized): Lyophilized preparation of gelatin particles sensitized with Inactivated HTLV-I Antigen. Reconsitute by adding prescribed amount of Reconstituting Solution at the time of use. Reconstituted particles contains 0.12% (w/v) of sodium azide as preservative.

D: Unsensitized Particles (Lyophilized): Lyophilized preparation of tanned gelatin particles. Reconstitute by adding prescribed amount of Reconstituting Solution at the time of use. Use this reagent as a control to check nonspecific agglutionation of specimens. Reconstituted particles contains 0.12% (w/v) of sodium azide as preservative.

E: Positive Control (Liquid):
The Positive Control (rabbit) is prepared to show the titer of 1:64 (a final dilution) when tested under the same test procedure used for specimens. This reagent contains 0.10% (w/v) of sodium azide as preservative.

Materials Provided:

1. Dropper approx. 25 μL 2 pcs.
Use exclusively for dispensing reconstituted Sensitized Particles and Unsensitized Particles.

Reconstitute Sensitized Particles and Unsensitized Particles 30 minutes prior to the test.

PREPARATION :

Prepare the following laboratory equipment before testing:

1) "U" shaped microplate
2) Diluters.....25 μL
3) Calibrated pipette droppers .....25 μL
4) Pipettes Micropipettes (with tips) .....25 μL and 50 μL for dispensing test samples
Volumetric pipettes .....1.0 mL, 5.0 mL and 10.0 mL for reconstitution
5) Droppers .....Approx. 25 μL (for adding reconstituted Sensitized and Unsensitized Particles)
6) Plate Mixer (automatic vibratory shaker)
7) Plate Viewer

TEST PROCEDURE:

Preparation of Specimens

Erythrocytes or other visible components present in the serum or plasma samples should be removed by centrifugation prior to testing in order to preclue interference with test results.

Test Performance:

[Qualitative (screening) Test Procedure] (see Table 1)

1) Place 1 drop (25 μL) of Sample Diluent in wells #1 through #3 using a calibrated pipette dropper.
2) Add 25 μL of serum/plasma specimen to well #1 using micropipette, and mix by filling and discharging the micropipette 3 or 4 times with fluid in well #1. Then fill the micropipette with 25 μL of diluted solution in well #1 and trarnsfer it into well #2. Mix well and transfer into well #3 following the same procedure as well #1. Repeat this procedure again in well #3 to obtain 2nd dilution.
3) Place 1 drop (25 μL) of Unsensitized Particles in well #2 and 1 drop (25 μL) of Sensitized particles in well #3 using the droppers supplied in the kit.
4) Mix the contents of the wells thoroughly using a plate mixer (automatic vibratory shaker). Then cover the place it on a level surface and allow to stand at room temperature (15 - 25 °C) for 2 hours. Afterwards read the patterns.

Table 1 Qualitative Test Procedure

Well No.		1	2	3	
Sample Diluent	(μL)	25 →	25 →	25 →	
Specimen	(μL)	25	25	25	discard
Specimen Dilution		1 : 2	1 : 4	1 : 8	
Unsensitized Particles	(μL)		25		
Sensitized Particles	(μL)			25	
Final Dilution			1 : 8	1 : 16	
Mix using a plate mixer (automatic vibratory shaker), cover plate and incubate for 2 hours.					
Interpretation					

[Performing Test using Positive Control] (See Table 2)

Table 2 Performing Positive Control Test

Well No.	1	2	3	4	5	
Sample Diluent (μL)	25 →	25 →	25 →	25 →	25 →	
Positive Control (μL)	25	25	25	25	25	discard
Specimen Dilution	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	
Unsensitized Particles (μL)						
Sensitized Particles (μL)			25	25	25	
Final Dilution		1 : 8	1 : 16	1 : 32	1 : 64	
Mix by using a plate mixer (automatic vibratory shaker), cover the plate and incubate for 2 hours.						
Interpretation						

Controls

1) Confirm that the reaction of each specimen and Unsensitized Particles (1:8 final dilution) is negative (−).
2) The mixture of Sample Diluent either with Sensitized Particles or Unsensitized Particles should give no reaction (−) for each run (reagent control).
3) Confirm that the titer of the Positive Control is 1:64 at final dilution when you perform the test procedure (see Table 2) for lot to lot consistency.

Interpretation

Place the microplate gently on a plate viewer (with indirect lighting), compare the negative agglutination patterns with those of the Reagent Control and interpret according to the criteria shown in Table 3.

Table 3

Settling Pattern of Particles	Reading	Interpretation
Particles concentrated in the shape of a button in the center of the well with a smooth round outer margin	(−)	Negative
Particles concentrated in the shape of a compact ring with a smooth round outer margin	(±)*	Inconclusive
Definite large ring with a rough multiform outer margin and peripheral agglutination	(+)	Positive
Filmy agglutinated particles spread out covering the bottom of the well uniformly	(++)	

*Note 3) Specimens which show an inconclusive result (±) should be retested following the Table 1 Test Procedure and test results shall be interpretted according to the criteria in Table 3. A repeated ± should be confirmed by other methods for acurate interpretation.

Criteria for Interpretation

In the test performance, a sample that shows a negative reaction with Unsensitized Particles (final specimen dilution, 1:8) but shows agglutination with Sensitized Particles (final specimen dilution, 1:16 or more) is regarded as giving a positive reaction.

Absorption Procedure

If a test sample causes agglutination with both Unsensitized and Sensitized Particles, it should be retested after the following absorption procedure.

1) Place 1 drop (25 μL) of Sample Diluent in well #3 of a microplate.
2) Place 0.3 mL of reconstituted Unsensitized Particles in a small test tube.
3) Add 0.1 mL of specimen, mix thoroughly using a tube mixer and inclubate at room temperature (15 - 25 °C) for 20 minutes (mix well by shaking manually 1 or 2 times).
4) Centrifuge for 5 minutes at 2,000 rpm. Separate the supernatant carefully

(absorbed specimen dilution, 1:4) and place 50 μL in well #2 of the microplate.
5) Mix by filling and discharging of a micropipette 3 to 4 times with fluid in well #2. Then fill the micropipette with 25 μL of diluted solution in well #2 and transfer it into well #3. Repeat this procedure in well #3 to achieve 2^n dilution.
6) Then follow the same test procedure as for the Qualitative Test Procedure and read the results.

PRECAUTIONS:
Safety Precautions
Sodium azide: NaN_3 0.12% (w/v)
EUH032: Contact with acids liberates very toxic gas.

Procedural Precautions
1) Mix the contents of the microplate wells thoroughly to ensure good test results.
2) During incubation, cover the microplate and avoid vibration.
3) All used instruments and tools (e.g., pipettes and tubes), waste solutions, tips, papers, etc. should be handled carefully, because they may be contaminated with HB or other viruses. Decontamination with glutaraldehyde solution (for at least 1 hour, with 2% solution), sodium hypochloride solution (Effective Chlorine concentration 1,000 PPM at least 1 hour), autoclaving (at 121 °C for at least 1 hour) or incineration is required, since specimens may be contaminated with HBs antigen.
4) In principle, lyophilized reagents contained in the kit should be used within the very day of reconstitution. However under storage at 2 - 10 °C they will remain stable for 7 days after reconstitution.

Handling Precautions
1) Although virus antigen coated onto the particles is an uninfectious substance, it should be treated carefully like HB virus.
2) Positive Control contained in this kit is of HTLV-Ⅰ immunized rabbit serum.
3) Sodium azide is used as preservative for reagents of SERODIA・HTLV-Ⅰ. Drain waste fluid with a large quantity of water for safety.
4) Please note that agglutination tests, in general, may on rare occasions exhibit a prozone phenomena. It has also been reported, that in rare cases, viral infection may not generate antibody production at some stage of infection.

[Preservation]
Store at 2 - 10 °C. Do not freeze.

[Shelf Life]
Refer to the outer package for expiry date. (The shelf life of the SERODIA・HTLV-Ⅰ test kit is 12 months after manufacture.) Please refer to the description appeared on the package and the labels on each vial.

[Contents]
Product No. 203132: Reagents for 100 qualitative.
Product No. 203170: Reagents for 220 qualitative.
Product No. 203248: Reagents for 550 qualitative.

[Manufacturer]
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