Leibniz-Institut
DSMZ-Deutsche Sammlung von
Mikroorganismen und Zellkulturen GmbH

Detection of Cassava brown streak virus & Uganda Cassava brown streak virus using monoclonal antibodies in TAS-ELISA

This ELISA to detect U/CBSV is a triple antibody sandwich format, TAS-ELISA. It employs a polyclonal antiserum (IgG) for coating and monoclonal antibodies (MAb) for decorating of the virus coat protein. Since the monoclonal antibodies are not labeled, a secondary, animal species (mouse) antibody is used to react with the bound MAb. This anti mouse (RAM) antibody is labeled with alkaline phosphatase (AP) as reporter group.

To reach optimum detection of viruses and especially CBSV in cassava the protocol shall be followed as exact as possible.

The TAS-ELISA requires 2 additional incubation steps compared to DAS-ELISA protocols:

1. **A blocking step to prevent nonspecific reactions: Addition of skim milk blocking reagent after coating.**
2. **Detection of bound MAb by a secondary antibody RAM AP-conjugate**.

This TAS-ELISA for detection of U/ CBSV is based on polyclonal and highly specific monoclonal antibodies which react with all U/CBSV species, strains and isolates so far described.

Coating: DSMZ AS- 1153

Mab: DSMZ AS- 1153/1

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Recommendations:

Sample extraction

The choice of leaf material determines the results of ELISA.
CBSV concentration is highest in symptomatic leaves. In young non symptomatic leaf materials virus is beyond detection limit.

The best cassava leaf material to use in TAS-ELISA and the limits of detection is to be determined with field samples.

Leaf samples are ground in sample extraction buffer and used at dilutions 1:20 (w/v) or 1:50 (w/v).
It is not recommended to use higher concentrations of sap which may lead to high background.

Sample incubation and washing:

Leaf samples shall be incubated overnight at 4°C. The plates need to be washed thoroughly to completely remove residual plant debris leading to false reactions and subsequently tapped dry.

Volumes:

Volumes of sample and reagents in ELISA are 100 µl. As a matter of convention, a DSMZ ELISA set is calculated for 200 µl volumes/ well and 100 reactions/ microtitre plate. **Hence an ELISA set is sufficient for 200 reactions and 2 plates.**

Serological reagents:

All antibodies and conjugates are sensitive and need to be handled carefully. The material is very reactive hence only small amounts of sera are used in dilution with respective buffers. The serological reagents have been thoroughly quality tested and volumes shipped carefully checked. The vials are special vials with safe lids preventing any evaporation and loss of liquid reagent.

Very important:

Tubes containing reagent solutions need to be centrifuged for a few seconds to collect the contents, also from liquid trapped in the lids at the bottom of the tube. This is to guarantee that the complete volumes are recovered.
Always include negative and **positive controls** in ELISA to verify functionality of the assay.

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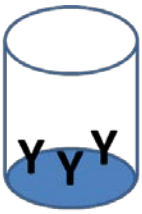
TAS-ELISA CBSV

Our ELISA reagents are optimized using greiner bio-one microplates, medium binding. Before opening the tubes containing Coating Antibody (IgG), MAb and RAM-AP- Conjugate please spin down all the liquid by a short centrifugation (approx. 3000rpm for a few seconds)



1. Dilute purified **IgG (AS-1153) 1: 250** in coating buffer (20µl in 10 ml buffer). Add 100 µl to each well of a microtitre plate.
2. Cover the plate and incubate at 37 °C for 2-4 h.
3. Wash plate with PBS-Tween using wash bottle, soak for a few minutes and repeat washing two times. Blot plates by tapping upside down on tissue paper.
4. Add 100 µl of 2% skim milk in PBS-Tween to each well (blocking). Cover the plate and incubate for 30 min at 37°C.
5. Remove blocking solution and tap dry.

6. Extract samples 1:20 (w/v) in sample extraction buffer . Add 200 µl aliquots of the test sample to duplicate wells.
7. Cover the plate and incubate overnight at 4 °C.
8. Wash three times as in step 3.

9. Add 100 µl of the **MAb (AS-1153/1) 1:50** in appropriate in conjugate buffer (40µl in 10ml buffer) to each well.
10. Cover the plate and incubate at 37 °C for 1 h.
11. Wash three times as in step 3.

12. Dilute RAM-AP 1:1000 in conjugate buffer (10µl in 10 ml buffer). Add 100 µl to each well.
13. Cover the plate and incubate at 37 °C for 1 hour.
14. Wash three times as in step 3.

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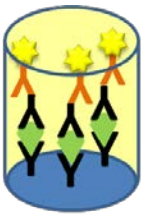

15. Add 100 µl aliquots of freshly prepared substrate (10 mg p-nitrophenyl phosphate [Sigma, Fluka] dissolved in 10 ml of substrate buffer) to each well.

16. Cover the plate and incubate at 37°C for 60 min and after 120 min and measure OD or assess results by visual observation.

Expected results :

Absorbance values in ELISA with CBSV free cassava leaves range between 0,05 - 0,15. An OD reading 3 times mean of all negative controls is rated positive in ELISA.

Buffers used in ELISA

1. ***Coating buffer (pH 9.6)***

   1.59 g sodium carbonate (Na_2CO_3)

   2.93 g sodium bicarbonate ($NaHCO_3$)

   0.20 g sodium azide (NaN_3)

   Dissolve in 900 ml H_2O, adjust pH to 9.6 with HCl and make up to 1 l.

2. ***PBS (pH 7.4) phosphate buffered saline***

   8.0 g sodium chloride (NaCl)

   0.2 g monobasic potassium phosphate (KH_2PO_4)

   1.15 g dibasic sodium phosphate (Na_2HPO_4)

   0.2 g potassium chloride (KCl)

   0.2 g sodium azide (NaN_3)

   Dissolve in 900 ml H_2O, adjust pH to 7.4 with NaOH or HCl and make up to 1 l.

3. ***PBS-Tween (PBST)***

   PBS + 0.5 ml Tween 20 per liter

4. ***Sample extraction buffer (pH 8.5)***

   0.05 M Tris containing 0.06 M sodium sulfite

5. ***Conjugate buffer***

   PBST + 2% PVP + 0.2% egg albumin (e.g. Sigma A-5253)

6. ***Substrate buffer***

   97 ml diethanolamine

   600 ml H_2O

   0.2 g sodium azide (NaN_3)

   Adjust to pH 9.8 with HCl and make up to 1 liter with H_2O

Buffers can be stored at 4 ° C for at least 2 months. Warm to room temperature before use.

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ELISA Troubleshooting

1. *No color development*

a) Did you omit any steps?

b) Did you use the correct buffer for each step?

c) Is your enzyme OK? Serum OK?

d) Is your positive control homologous to antiserum (IgG)?

Recommendations - Do a titration plate. Use reliable positive control in each plate. Pretest enzyme conjugate on substrate. Check purified IgG for antigen-specific IgG.

2. *Nonspecific color development*

a) If in edge wells only:

- Make sure the humidity in the incubator is sufficiently high.
- If this does not help, don't use edge or border wells, fill with buffer only.

b) If in whole plate:

- incomplete washing
- old substrate
- use recommended ELISA plate (greiner medium binding)
- error in loading sequence

Recommendations - Use reliable negative control in each plate.
Use fresh substrate and check for spontaneous color change. Cover plates while incubating. Check pH of the buffers used.

c) Some wells with inconsistent or unexpected reactions

- incomplete washing
- error in loading test antigens
- spillage between wells

Recommendations - Use extra wash step, handle plates carefully with lids on, use predetermined loading pattern before loading. Blot top of plate after rinsing.

3. *Color development very rapid; some color in healthy samples*

a) Enzyme conjugate concentration too high

b) Substrate concentration too high

Recommendations - Use enzyme conjugate and substrate concentrations that will give $OD_{405\ nm}$ of about 1.0 in 30 to 60 min with good antigen source.

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