Mercodia

Technical Note, No: 34-0108, v.4.0

Troubleshooting manual

1. High signal in some or all wells

Sources of error	Cause	Background/Suggestions
Contamination of substrate	Substrate was contaminated by enzyme conjugate solution.	Always use clean pipette tips, beakers etc. in order to avoid contamination. Rinsing with water is not enough. Containers and pipette tips used for enzyme conjugate solution should be kept separate from the substrate.
Contamination by samples or calibrators	Assay- or Sample Buffer was contaminated by samples or calibrators.	Always use clean pipette tips, beakers etc. in order to avoid contamination.
Washing procedure	Insufficient washing before substrate incubation.	Plate washer should be checked and served periodically. When washing manually use a laboratory wash bottle, see Tech note 34-0106 Instruction for manual washing.
Incorrect dilution of enzyme conjugate solution	Too much enzyme conjugate stock solution has been added to the Enzyme Conjugate Buffer.	Dilute enzyme conjugate solution according to Directions for Use.

2. No signal/absorbance in any well

Sources of error	Cause	Background/Suggestions
Stop Solution	Substrate TMB was contaminated by stop solution prior to addition to wells. The assay procedure has been done incorrectly.	Avoid contaminating Substrate TMB with Stop Solution by labeling any containers used. Always make sure that the stop solution is added after the Substrate TMB. The Stop Solution causes the enzyme-substrate reaction to reach end-point.
Enzyme conjugate solution	No enzyme conjugate stock solution was added to the Enzyme Conjugate Buffer.	Dilute conjugate according to the Directions for Use.
Wash Buffer	Wash Buffer containing Sodium Azide has been used instead of the wash buffer provided in the kit.	The Wash Buffer in the kit is optimized for use with Mercodia ELISA kits. Washing solution from other companies may contain Sodium Azide, which can deactivate the enzyme. We therefore recommend using the Wash Buffer included in the kit.

3. No signal in one or a few wells

Sources of error	Cause	Background/Suggestions
Calibrator, sample	No sample or Calibrator was added to the well.	It can, especially for assays with low sample volume, be difficult to see if Calibrator/Sample has been added to each well. Be careful not to miss a well when pipetting calibrators and samples.
Sodium Azide	Sample contains Sodium Azide.	Sodium Azide can deactivate the enzyme.

4. Low absorbance in all wells

Sources of error	Cause	Background/Suggestions
Plate reader lamp	The intensity of the ELISA plate reader lamp is too low.	Check lamp efficiency and plate alignment. This can be done according to the Tech note 34-0107 Instruction for plate reader.
Absorbance	Absorbance was read with another wavelength than 450.	Check that the wavelength is set to 450 nm and that the right filter is in the plate reader.
Plate shaker	Plate shaker amplitude is too low.	Plate shakers should have a speed of approximately 700-900 cycles per minute (rpm). Low frequency results in poor calibration curve.
Pipettes	Pipettes are not calibrated, resulting in low sample/calibrator volume.	Pipettes should be calibrated and cleaned regularly.
Incubation time	Incubation time is too short.	Do not prolong or shorten incubations.
Preparation of enzyme conjugate solution	Over dilution of enzyme conjugate solution.	Dilute enzyme conjugate solution according to Directions for Use.
Incorrect wash	Soak was included in the wash procedure	Program the plate washer to aspirate immediately after the dispensing of Wash Buffer. If washing manually hold the plate vertically. When washing manually use a squirt bottle, see Tech note 34-0106 Instruction for manual washing.

5. High backgrounds

Sources of error	Cause	Background/Suggestions
Washing	Insufficient washing.	Plate washer should be checked and served periodically. When washing manually use a squirt bottle, see Tech note 34-0106 Instruction for manual washing.
Contamination of Calibrator 0, Assay Buffer or Conjugate Buffer	Contamination of Calibrator 0, Assay buffer or Enzyme Conjugate Buffer with Calibrators, Controls or samples.	Always use clean pipette tips, beakers etc. in order to avoid contamination.
Contamination of substrate	Substrate might be contaminated with enzyme conjugate.	Containers and pipette tips used for enzyme conjugate solution should be kept separate from the substrate.
Substrate TMB	Substrate TMB has been exposed to light.	Substrate TMB is light sensitive. Uncovered Substrate can cause increased background if left out in the light for only a couple of minutes.
Dried solution on the bottom of the plate	Dried wash solution under the plate, on the outside of the wells.	Dampen a tissue with alcohol and carefully wipe off the bottom of the plate. Re-measure immediately.

6. High CV (coefficient of variation)

Sources of error	Cause	Background/Suggestions
Washing	Insufficient washing.	Plate washer should be checked and served periodically. When washing manually use a squirt bottle, see Tech note 34-0106 Instruction for manual washing.
Pipettes	Pipettes are contaminated or out of function.	Pipettes should be calibrated and cleaned regularly.
Dried solution on the bottom of the plate	Dried wash solution under the plate, on the outside of the wells.	Dampen a tissue with alcohol and carefully wipe off the bottom of the plate. Re-measure immediately.
Bubbles in the wells when the plate was read	Pipetting error	Use repeating or multi-channel pipettes when adding substrate and Stop Solution. Use reverse pipetting to avoid bubbles.
Greenish colour in the wells	Insufficient stop of Substrate TMB reaction	Mix the plate immediately after adding Stop Solution to the wells.
Plate shaker	Plate shaker amplitude is too low.	Plate shakers should have a speed of approximately 700-900 cycles per minute (rpm) orbital movement. Low frequency results in poor calibration curve.

7. Lower concentration than expected

Sources of error	Cause	Background/Suggestions
Reconstitution volume (for kits with lyophilized calibrators or controls)	The lyophilized calibrators have been reconstituted with a smaller volume than stated in the Directions for Use.	Reconstitute with the correct volume of redistilled water as stated in the Direction for Use. Mix well before using. Check that there is no un-dissolved material left on the stopper of the bottle.
Hook effect	The sample contains a very high amount of antigen and is therefore given a falsely low result because of the hook effect.	Dilute and re-run the samples.
Dilution	The samples and/or controls have been diluted too much.	Always use the volumes stated in the Direction of Use for samples, calibrators, controls and enzyme conjugate solution. Check that pipettes are calibrated.

8. Higher concentration than expected

Sources of error	Cause	Background/Suggestions
Reconstitution volume (for kits with lyophilized calibrators or controls)	The lyophilized calibrators have been reconstituted with a larger volume than stated in the Directions for Use.	Reconstitute with the correct volume of redistilled water as stated in the Direction for Use. Mix well before using.
Dilution	The samples and/or controls have not been diluted enough.	Always use the volumes recommended for samples, Calibrators, Controls and enzyme conjugate solution. Check that pipettes are calibrated.

9. General advice

When running assays with low sample volume, ≤10 µL, please make sure that the pipette used is designed and calibrated for the specific volume. Reverse pipetting is recommended for small volumes, viscous liquids and for liquids that foam easily.

You may always contact Mercodia for personalized assistance. Please send an email to support@mercodia.com.