Technical Hints

M30 Apoptosense® ELISA (Prod. no. 10011)

For Research Use Only

Kit storage

Store the M30 Apoptosense® ELISA at 2-8 °C.

Sample storage

At 2 – 8 °C	Up to 4 hours
At -20°C or lower	For longer periods

Sample preparations

Sample type

One single type of samples, e.g. serum or plasma, collected by one method should be used for a specific project.

Note: Use tubes without anti-coagulant when collecting serum samples.

Dilution of blood samples

Samples with a concentration higher than 1000 U/L (i.e. higher than the highest Standard) should be diluted using either Standard A or blood donor serum.

With Standard A up to 1:10
With Serum up to 1:50

See the graph under section "Reproducibility" for more information about CV values on samples with a concentration below 200 U/L

The original concentration in the assay is calculated by multiplying the measured concentration with the dilution factor, using the formula below. When using blood donor serum as a diluent, the concentration (U/L) of the blood donor serum must be accounted for.

$$((c1 \times v1) + (c2 \times v2))/ (v1+ v2)$$

c1 = conc. of sample undiluted
c2 = conc. of diluent or blood donor sample
v1 = volume of sample
v2 = volume of diluent or blood donor sample

% Recovery = Obtained value/Expected value x 100

Kit preparations and performance

Preparing the reagents

Reagents as well as samples should be allowed to reach a room temperature of 24 ± 3 °C and be vortexed prior to use.

Note: Do not mix reagents from different kit lots.

Dispensing the reagents

The addition of reagents and samples to the plate should be performed without interruption within 20 minutes.

Note: Avoid contamination between the wells when dispensing the reagents and samples.

Preparing and splitting up the M30 Conjugate

Dilute the M30 Conjugate with the M30 Conjugate Dilution Buffer to prepare the M30 Conjugate solution.

The M30 Conjugate, diluted or undiluted, is sensitive to light and should be stored in the original amber bottle between uses, at 2 – 8 °C.

Using all the M30 Conjugate
If the kit is used at one single occasion, add the M30 Conjugate Dilution Buffer to the M30 Conjugate vial and mix.

The diluted M30 Conjugate solution is stable for 3 weeks, at 2 – 8 °C.

Splitting up the M30 Conjugate
If the kit is used at several occasions, split up the M30 Conjugate by using the table below. When splitting up the M30 Conjugate, mix the M30 Conjugate and the M30 Conjugate Dilution Buffer in a separate container.

For stability of the undiluted M30 Conjugate components, see the expiry date on the bottle.

Number of strips	M30 Conjugate (µL)	M30 Conjugate Dilution Buffer (µL)
3	90	2070
6	175	4025
9	250	5750
12	400	9200

If not all the strips are used, store the remaining strips in the sealed aluminium bag and keep the desiccating device inside.

Wash tablet

Dissolve one Wash Tablet in 500 ml of fresh deionised water.

The Wash Tablet solution is stable for 5 weeks when stored at 2 – 8 °.

Note: Additional Wash Tablet can be ordered separately (Product number 20500)

Washing step

To remove unbound M30 Conjugate, wash the plate using a multichannel pipette or a 96-well microtiter plate washer. Avoid contamination between the wells.

Manual washing – Remove the incubation solution and wash the plate five times with Wash Buffer (a minimum of 250 µL/well).

Automated washing - Wash the plate five times with Wash Buffer (400 – 500 µL/well).

After the final wash, discard the solution and tap the inverted plate on an absorbent surface to remove all solution.

Proceed to the next step immediately after the last washing round in order to avoid drying the wells out.

TMB Substrate

The TMB Substrate is very sensitive to light and temperature. Do not leave the bottle at room temperature during prolonged periods.

TMB Substrate cannot be used after exposure to light. If the kit is used at several occasions, take out only the necessary amount of TMB Substrate from the amber bottle at each occasion.

Stop solution

To ensure good mixing when dispensing the Stop solution, insert the pipette so that the tip is submerged, tilt to create an angle and empty the pipette firmly. Adding the Stop solution in this way will create a whirl, mixing the reagents in the well without the appearance of bubbles.

Shake the plate gently for 5-10 seconds after adding the Stop solution and incubate at room temperature for 5 minutes. Avoid exposing the plate to direct sunlight during incubation.

If any bubbles would remain after the incubation make sure to remove these, by using e.g. a needle or a pipette tip, before measuring the absorbance.

Calculation of analytical results

Recommended method

For the data analysis, the fitting algorithm Cubic spline is recommended.

Cubic spline was used during verifications of the kit and all values and concentrations defined in the Instructions for Use were generated and validated using Cubic spline.

Fitting algorithm:	Cubic spline
X-axis:	concentration (Units/Liter)
Y-axis:	absorbance (OD at 450 nm)

Alternative method (For Research Use Only)

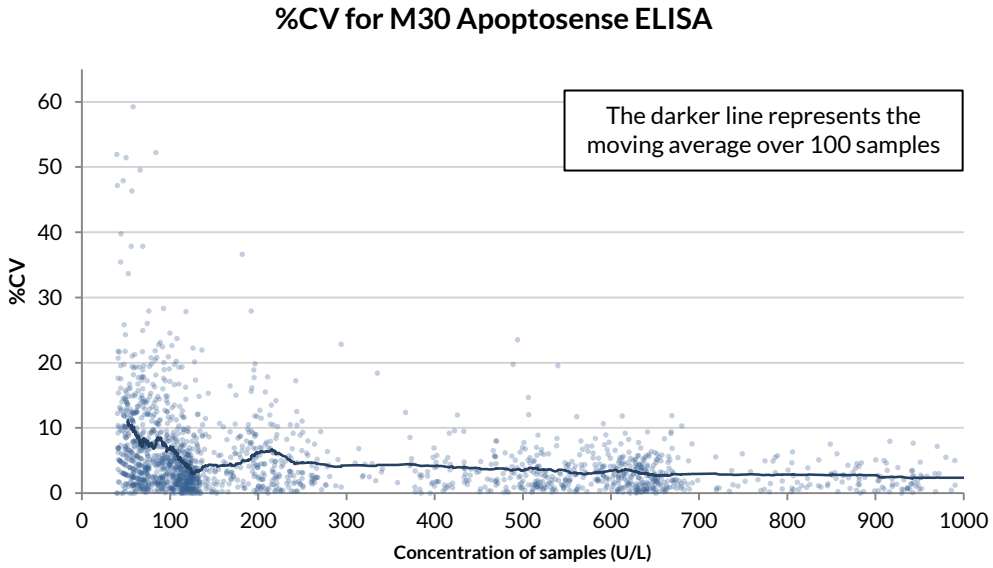
If Cubic spline is not available, other fitting algorithms can be used. One example of such algorithm is 4PL. When using 4PL the calculated concentrations will differ from results analysed with Cubic spline.

Validations, verifications and QC controls of the M30 Apoptosense® ELISA are performed using Cubic spline and the data presented in the Instructions for Use and in this document were obtained using Cubic Spline. If an alternative algorithm is used, the manufacturer disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and the fitness for use.

Reproducibility

Samples above 200 U/L - Within assay (WA %CV) variation is ≤ 10 %, between assay (BA %CV) variation is ≤ 10 % and total variation is ≤ 10 %

Below is a graph showing the %CV over duplicate wells of 1871 measurements obtained in 3 lots of M30 Apoptosense ELISA, 10011, during its analytical verification.



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VLVbio
Hästholmsvägen 32
131 30 Nacka
Sweden

Phone +46 8 122 053 00
E-mail: info@vlvbio.com