

GUIDELINES FOR PUTTING AUTOZYME™ ASSAYS ON AUTOMATED ANALYSERS

These are guidelines to complement the relevant 'Instructions for Use' (IFU) booklet for the Cambridge Life Sciences AUTOZYME™ range of Elisa assays, which has been validated by Cambridge Life Sciences. The guidelines and the relevant IFU should enable the user to set the instrument parameters to run on automated Elisa analysers.

Sample

Human serum or plasma may be used (refer to section 6, Sample Handling of the IFU). Check with the analysers IFU if the samples can be used directly from the blood collection tubes or decanted in to new tubes. The minimum sample volume for most analysers is 200µL.

Reagent Preparation

Dilute the contents of the Wash Buffer concentrate vial to 1 Litre with RO/deionised water unless stated otherwise by the relevant IFU (refer to section 9, Assay Procedure). Decant the diluted wash buffer in to the relevant wash buffer container for the analyser and prime the analyser to ensure that water or the previous wash buffer does not come in contact with the kit microplate.

Do not dilute calibrators or controls provided with the kit. Some analysers will take the reagent bottles others will require the careful decanting of reagents in to generic reagent containers. Remember to remove the caps from the bottles. If decanting, label the generic containers and seal with the lids provided if not using the whole kit. Unused reagents and reconstituted wash buffer are stable for 1 month after first opening the container. Multiple re-use could increase the risk of contamination. Keep all reagents away from direct sunlight and store refrigerated at 2 – 8°C until required. Allow a minimum of two hours to equilibrate to room temperature before use.

Assay Procedure

Depending on the order for the analyser, set the following parameters;

- Sample, conjugate, substrate and stop volumes as directed on the relevant IFU. For AUTOZYME™ assays these are usually 100µL but there are some exceptions.
- Sample, conjugate and substrate incubation times as directed on the relevant IFU. There maybe an option for setting the incubation temperature, generally this is left off to allow room temperature incubation. The incubation should be without shaking. We recommend a 10 sec shake step after the sample pipetting step to ensure the samples are uniformly distributed in the wells and also after the stop pipetting step before the plate is read to ensure the stop has mixed with the substrate solution.
- Sample dilution as directed on the relevant IFU. Our assays generally use 1/100 dilutions and the samples are normally prediluted into tubes by the analyser before dispensing in to the microplate. 10µL sample plus 990µL sample diluent are recommended. Accuracy is generally reduced for pipetting volumes below 10µL. Do not dilute sample diluent in to the microplate and then pipette in the sample for an in-well dilution as this does not give good results.



- Wash cycles. 3 x 300µL by strip full with 15 sec soak time after the sample incubation and 0 sec soak time after the conjugate incubation is recommended for automated analysers. By strip full means the first strip is washed 3 times and then left with 300µL wash buffer in the wells before proceeding to the next strip. After completing all the strips a final aspirate is done on the whole plate. This prevents the wells drying out.

The analysers generally take 15 minutes to pipette a whole plate of calibrators and controls, the wash cycle with a 0 sec soak time takes approx 4 minutes to wash the plate, therefore there is approx 10 minutes in a 30 minute sample incubation period difference between column 1 on the plate and column 12 on the plate which can lead to differences in results between the start and end of the plate. Adding a soak time of 15 sec removes this difference.

It is not required after the conjugate incubation as the dispense time for conjugate and substrate is approx the same time as the wash cycle with 0 sec soak. For conjugate, substrate and stop solutions the analyser takes a larger volume and dispenses usually in to all the wells of a column in one go. The soak time will need to be checked for the particular analyser depending on its pipetting times and also if a different number of wash cycles are used.

Note - The analyser may need to be adjusted for the specific microplates used in the AUTOZYME™ assays. Always check the performance of a microplate washer with a coated microplate and proper wash buffer as uncoated wells and water do not aspirate in a representative way due to the hydrophobic nature of the uncoated microplate. Minimal wash buffer should be left in the wells after a wash cycle, < 2µL liquid.

- Wavelength. Set the wavelength as directed on the relevant IFU. There is no requirement to use dual wavelength. For ABTS substrates where the primary wavelength is 405nm, it is important that 620nm is **not** used as a secondary wavelength. ABTS produces a green/blue colour and 620nm measures blue which for ABTS can reduce the OD by as much as 50%. If a dual wavelength is required set the secondary wavelength to 492nm.

Calculation of Results

Quantitative Assays

Assays that have a number of calibrators (5 or more) are recommended to use the 4-parameter fit programme on the analyser. Set the calibrator values to those stated in the relevant IFU.

Qualitative Assays

Assays that have a single calibrator are recommended to use the cut-off programme on the analyser. Set the calibrator value to those stated in the relevant IFU. The following algorithm is generally used to calculate the sample ratio or concentration:

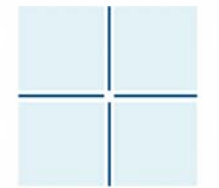
$$\frac{\text{Calibrator concentration}}{\text{Calibrator OD}} \times \text{Sample or control OD} = \text{units}$$

Quality Control

Good laboratory practice requires that quality control specimens be included in every run to monitor assay performance (refer to section 11, Quality Control of the IFU). Kit controls are supplied with every AUTOZYME™ kit and we make every effort to keep the control ranges constant. The control ranges are found on the Certificate of Analysis supplied with the kit. Please check the Certificate of Analysis to confirm the control ranges to be entered on the automated analyser.

Important

Do not substitute kit reagents or mix reagents from different kit lot numbers. Cambridge Life Sciences recommend the NexGen Four automated analyser from Adaltis and the AP Speedy analysers from DAS. Contact Cambridge Life Sciences for full details of these analysers and protocols.



CAMBRIDGE
LIFE
SCIENCES

