Always use the package insert as the reference.

General preparation
- Bring all reagents and the aluminum foil bag containing the strips to room temperature (18-30°C), approximately 60 min before use.
- To avoid water condensation into the wells, the aluminum foil bag must be kept closed until the strips have reached room temperature.
- Allow ready-to-use calibrators (CAL), Run Validation Controls (RVC) and CSF samples to reach room temperature (18-30°C) approximately 60 min before use.
- Put the Wash Solution in an incubator or warm water bath at 30-40°C for 60 min to dissolve salt crystals.

Preparation of the Conjugate working solution 1:
- Make a 1:100 dilution of the concentrated Conjugate 1 in Conjugate Diluent 1 (red color).
- Dispense 75 µl Conjugate working solution 1 into the coated microplate wells.

Preparation plate:
! We advise the use of this preparation plate when more than 6 strips need to be used. Vortex CAL, RVC and samples for 10 seconds and dispense ≥ 60 µl of sample/CAL/RVC into the wells of the preparation plate.

From this plate, 2 x 25 µl CAL, RVC and samples need to be transferred into the coated INNOTEST β-AMYLOID(1-42) plate. This action will reduce a reactivity shift.

Dispensing CAL, RVC and samples into the coated INNOTEST β-AMYLOID(1-42) plate:

a) In case a limited number of samples needs to be tested:
Add 25 µl of each sample/CAL/RVC to duplicate wells of the antibody-coated plate.

b) In case a larger number of sample needs to be tested (more than 6 strips):
Use a multichannel pipette to transfer 25 µl from each well of the preparation plate to duplicate wells of the antibody-coated plate.

- Mix the fluids by tapping the side of the plate by hand or by shaking the plate 1 min at 1000 rpm.
- Cover the plate with a plate sealer.
INNOTEST® β-amyloid(1-42) 
(2/2)

**INCUBATION**: 60 ± 3 min ⊕ at 25 ± 2°C in an incubator

**Preparation of the diluted Wash Solution**

<table>
<thead>
<tr>
<th>Wash Solution:</th>
<th>1 strip (8 wells)</th>
<th>12 strips (96 wells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash Solution 25x</td>
<td>5 ml</td>
<td>60 ml</td>
</tr>
<tr>
<td>H₂O</td>
<td>120 ml</td>
<td>1440 ml</td>
</tr>
</tbody>
</table>

**Preparation of the Conjugate working solution 2** just before the end of the sample incubation:
- Make a 1:100 dilution of concentrated Conjugate 2 in Conjugate Diluent 2 (green color).

**Wash procedure** – automatic and manual:
- aspirate the CAL/RVC/sample + Conj. working solution 1
- invert the plate on a tissue and tap dry
- dispense 400 µl washing solution into each well, soak 30 seconds
- aspirate the washing solution
- invert plate on an absorbent tissue and tap dry

**Dispense 100 µl Substrate working solution into the wells.**

**INCUBATION**: 30 ± 3 MIN ⊕ at 25 ± 2°C in an incubator IN THE DARK

**Stopping the reaction:**
- Add 50 µl Stop Solution to each well.
- Mix the fluids by tapping the side of the plate by hand or by shaking the plate 1 min at 1000 rpm.

**Reading:**
- Read the absorbance at 450 nm (single wavelength) within 15 minutes after addition of the Stop Solution.
- For dual wavelength analysis, 620 nm can be used as the reference wavelength.