Always use the package insert as the reference.

General preparation Day 1
- Bring the coated plate, Sample Diluent, Conjugate Diluent 1 and Conjugate 1 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.

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.

Dispensing CAL, RVC and samples into the coated INNOTEST hTAU Ag plate:
- Vortex CAL, RVC and samples for 10 seconds and add 25 µl CAL, RVC and sample to the coated plate wells.
- Mix the fluids gently 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.

General preparation Day 2
- Bring the Wash solution, Conjugate Diluent 2, Conjugate 2, Substrate, Substrate Buffer and Stop Solution to room temperature, 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 diluted Wash Solution
<table>
<thead>
<tr>
<th>Wash Solution: 25x</th>
<th>1 strip (8 wells)</th>
<th>12 strips (96 wells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>5 ml</td>
<td>60 ml</td>
</tr>
<tr>
<td></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 or 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 Conjugate working solution 2 into the wells.
- Cover the plate with a plate sealer.

INCUBATION: 30 ± 3 MIN ⊗ at 25 ± 2°C in an incubator

INCUBATION: 14 to 18 hours ⊗ at 25 ± 2°C in an incubator
INNOTEST® hTAU Ag
(2/2)

Preparation of the Substrate working solution just before the end of the conjugate incubation:
- Make a 1:100 dilution of concentrated Substrate in Substrate Buffer.

Wash procedure – automatic and manual:
- aspirate the Conj. working solution
- 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 a 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.