



# Rabbit brain acetone powder clotting time control

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FR

## 1 - PRESENTATION

The following example is given for a quantity of initial Rabbit Brain Acetone Powder of about 1.5 grams.

*Frozen powder samples have to be set at room temperature during at least 30 minutes before their use.*

Note: Take care to avoid re-moisturization of the powder.

- Place 10 mL of physiological sera in a 15 mL tube and preheat in a 37°C water bath for at least 20 minutes. Add 1.5 grams of powder and homogenize the solution by shaking up and down vigorously.
- Incubate 23 minutes at 37°C while shaking up and down vigorously every 3 minutes. This time instruction (23 minutes) has to be scrupulously followed for each powder sample; so if there are several samples to treat, the first one is put in the water bath at T<sub>0</sub>, the second one at T<sub>0</sub>+30s, the third one at T<sub>0</sub>+1min, etc... Then the same order must be respected when the tubes are taken out of the water bath.
- Centrifuge at 2465 g for 15 minutes at room temperature (3000 rpm with a rotor of 24.5 cm radius).
- Collect 0.5 mL of supernatant (thromboplastin) in the middle of the upper phase (take care not to mix the pellet with particles).

Extraction yield will vary depending on extraction conditions, such as:

- The extraction temperature (do not exceed 42°C to prevent damage of extracted thromboplastin. The optimal extraction temperature is between 37 and 42°C),
- The ionic strength of the extraction medium (an optimal ionic strength must be determined for optimum extraction yield),
- Addition of specific chemicals (for example: the addition of 7.5 mmol/L of calcium formate during the extraction step may contribute to obtain a thromboplastin more sensitive to factor VII).

## 2 – DETERMINATION OF EXTRACTED THROMBOPLASTIN CLOTTING TIME

- Mix the supernatant as follows:
  - 0.5 mL thromboplastin,
  - 1.6 mL CaCl<sub>2</sub> (0.025 M),
  - 1.9 mL of distilled or desionized water.
- Perform the thromboplastin clotting time on fresh citrated plasma or lyophilised plasma according to the usual procedure (*1 volume of plasma for 2 volumes of thromboplastin*).
- Plasma:
  - Clotting time is controlled on undiluted plasma and 1:4 diluted plasma.
  - Plasma has to be set at room temperature at least 30 minutes before use.
  - For lyophilised plasma, mix with 1 mL of purified water and then stabilize at room temperature during 30 minutes.
  - Keep a part of this solution for the control on undiluted plasma and dilute a part of the solution with Owren-Koller buffer for the control on 1:4 diluted plasma.
  - This solution is stable during 4 hours at room temperature.
- Thromboplastin:
  - Thromboplastin has to be incubated during 20 minutes at 37°C before the tests.
- Neoplastin:
  - Neoplastin has to be set at room temperature at least 30 minutes before use.
  - Mix the solvent with the lyophilized solution.
  - Stabilize at room temperature during 30 minutes.
  - Shake up and down slowly in order to obtain an homogenous suspension.
  - Incubate the solution during 20 minutes in a 37°C water bath before the tests on ST4 or STart Instrument.
  - This solution is stable during 8 hours at 37°C, 24 hours at room temperature, 8 days at 2-8°C.
- Tests (2 internal references are used for positive control):
  - Put magnetic beads for STart Instrument in cups for STart Instrument.
  - Prepare the STart Instrument.
  - Stabilize the temperature of the STart at 37°C.
  - Place the sample to test in a tube in the Start Instrument with an Eppendorf pipette.
  - Place 50 µL of undiluted plasma or 1:4 diluted plasma in cups.
  - Incubate during 120 seconds in the Start Instrument.
  - 10 seconds before the end :
    - put the Start Instrument in measure position,
    - homogenize the thromboplastin with a pipette,
    - put 100 µL of the thromboplastin in the cup.
  - Record the clotting time.

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