

#### PRODUCT DESCRIPTION

UPTT Reagent is a lyophilized preparation of buffered rabbit brain cephalin that has a standardized phospholipid concentration of  $\leq 0.1\%$ . The UPTT Reagent **does not** contain activating compounds.

#### INTENDED USE

UPTT Reagent is intended for use in performing (non-activated) in-vitro Partial Thromboplastin Time (PTT) tests. This test detects the activating effects of a device or extract on the intrinsic coagulation pathway in platelet poor or platelet free plasma<sup>1,2</sup> or the deficiency of certain plasma coagulation factors.<sup>1,2,3,4</sup>

#### PRINCIPLE

Contact activation occurs in the absence of  $Ca^{++}$  following whole blood exposure to a foreign material.<sup>4,5,6</sup> The UPTT measures the plasma factors which are involved in the generation of plasma thromboplastin via the intrinsic pathway by detecting the time to clot formation.<sup>7,8</sup>

The test should be run in triplicate. Compare the results following exposure to the test device to those on the untreated plasma and negative controls.

#### PRECAUTIONS

UPTT reagent is **not** for use in human or animal diagnostic tests or procedures.

Observe Standard Precautions throughout reagent preparation, specimen collection, sample preparation and analytical processes.<sup>9</sup>

Dispose of sharps and biological waste in accordance with laboratory policies.

#### MATERIALS PROVIDED

UPTT Reagent 20 x 3.0mL

#### REAGENT STORAGE

Lyophilized UPTT Reagent - Store at 2 - 8° C until expiration date.

Reconstituted UPTT Reagent is stable for seven days when stored in the tightly sealed original container at 2 - 8 °C.

#### MATERIALS REQUIRED BUT NOT PROVIDED

1. Purified Water, pH 5.3 - 7.2 (distilled, deionized or reagent grade)
2. Pipettes (10.0, 3.0 and 0.1mL volume capacities).
3. Calcium Chloride, 0.025 M (C/N 100989)
4. Sodium Citrate, 0.11M (C/N 100994)
5. Coagulation control plasma

#### RECONSTITUTION

Warm vial to room temperature prior to reconstitution.

1. Tap vial to dislodge material adhering to stopper
2. Remove the aluminum seal
3. Remove the stopper and reconstitute the vial contents with 3.0 mL of purified water.
4. Replace the stopper and invert the vial to thoroughly mix the contents. Let stand for not less than 15 minutes prior to use to assure complete rehydration of the contents.

Once reconstituted, UPTT is stable for seven days when stored in the tightly sealed original container at 2 - 8 °C.

#### COLLECTION AND PREPARATION OF TEST PLASMA

Test samples for UPTT **must** be platelet free or platelet poor plasmas prepared from anticoagulated whole blood.<sup>2,11,12</sup>

#### Specimen Collection

Blood collection should be performed with care. Release the tourniquet once the blood is visible in the collection device. Avoid stasis, hemolysis, contamination by tissue fluids, or exposure to glass.

#### Evacuated Specimen Collection Tube Technique (recommended)<sup>12</sup>

1. Use a winged needle collection set approved for laboratory specimens
2. Draw blood into the plastic, evacuated specimen collection tube containing 0.11M sodium citrate anti-coagulant

3. Mix the specimen by gently inverting the tube 4-5 times

**NOTE:** Verify the anticoagulant type and concentration being used. Colored tops **do not vary** with differing citrate concentrations.

#### Syringe Technique (alternate procedure)<sup>13</sup>

1. Use a winged needle collection set approved for laboratory specimens
2. Draw 9.0mL of blood into a plastic syringe. Avoid excess suction.
3. Remove the needle from the collection set
4. Immediately and gently dispense the blood into a polypropylene tube containing 1.0mL of 0.11M sodium citrate (9:1 blood to anticoagulant ratio)
5. Cap the tube and gently invert 4 -5 times to mix.
6. Maintain specimens for a maximum of two hours at room temperature prior to testing.

#### Sample Preparation

#### PDQ® Platelet Function Centrifuge

1. Place the specimen in the PDQ
2. Select the PFP setting
3. Push Start

#### Bench Top Centrifuge: Platelet Poor Plasma Preparation<sup>12,14,15,16</sup>

1. Place specimen in the centrifuge
2. Centrifuge specimen at 2500 x g for 15 minutes.

#### Harvest Sample

1. Use a plastic transfer pipette
2. Remove platelet poor plasma carefully; do not disturb the buffy coat.
3. Transfer sample to a plastic tube and cap
4. The sample can be stored at (15 - 28°C) for a maximum of two hours before testing

- Use of a discard tube should be in accordance with laboratory policy.
- Adjust specimen volume for a 9 to 1 blood anti-coagulant ratio based on the specimen's hematocrit. Adjustment required for hematocrits <30% or >55%.<sup>7,12,17</sup>
- Do not expose the whole blood specimen or test sample to glass at any point during the procedure.<sup>14,16</sup>
- Do **not** perform UPTT on samples that are visibly hemolyzed, icteric or lipemic.<sup>12</sup>

**NOTE: For best results, suspension of the UPTT Reagent should be maintained either by magnetic stirring or gentle inversion immediately prior to use. Use a plastic coated, disposable stir bar.**

#### TEST PROCEDURES

#### INSTRUMENTATION

Partial Thromboplastin Time endpoints can be detected by manual coagulation methods, and by most coagulation analyzers. The analyzer test method is the same as the one used to perform **activated** Partial Thromboplastin Time Tests (aPTT). Follow the manufacturer's instructions for performing the assay. The laboratory established UPTT reference range **must** be used.<sup>10</sup>

- All tests should be performed in triplicate.
- A 1.0 gram sample is required for devices with indirect blood contact.
- A 2.5 gram sample is required for devices with direct blood contact.
- The amount of time that the device under evaluation is in contact with the test plasma must be precisely controlled.

#### MANUAL

**NOTE: This test procedure is for manual methodology. For use with automated or semi automated analyzers, follow the instructions in the analyzer's operations manual.**

1. Pipette 0.1mL test or control plasma into a test cuvette and incubate the plasma for 2 minutes at 37° C.
2. Pre-incubate 0.025 M Calcium Chloride at 37° C.
3. Pipette 0.1mL UPTT Reagent into the test cuvette containing the plasma.
4. Incubate the plasma/reagent mixture for 2 to 5 minutes at 37° C or as specified by your protocol.
5. Add 0.1mL of the pre-incubated calcium chloride, simultaneously starting a timer.
6. Record the clotting time

- All tests should be performed in triplicate
- A 1.0 gram sample is required for devices with indirect blood contact
- A 2.5 gram sample is required for devices with direct blood contact
- The amount of time that the device under evaluation is in contact with the test plasma must be precisely controlled

#### QUALITY CONTROL

Laboratories should follow generally accepted quality control practices. Controls should be run each day the test is performed.

1. Two levels of negative controls must be used for each device sample tested
2. One positive control must be run for each device sample tested

If a coagulation analyzer is used to perform the UPTT, the manufacturer's directions for the proper operation, quality control and maintenance must be followed.

Each laboratory should determine acceptance limits for each lot of UPTT Reagent and control plasmas used by performing replicate studies in accordance with established laboratory procedures. Comparison to **Activated** Partial Thromboplastin Time (APTT) results run in the same fashion may be used.

Each laboratory **must** determine reference ranges and acceptance limits for each lot of UPTT Reagent and control plasmas.<sup>10</sup> Crossover studies are required when the UPTT Reagent lot number changes.

#### ACCURACY, PRECISION AND REPRODUCIBILITY

**The CV for normal plasma and control levels 1, 2, and 3 should be less than 5%.**

The precision and accuracy of UPTT test results may be influenced by a number of factors. Significant protocol and inter-laboratory differences arise from the number of coagulation systems used to measure the clotting end point.<sup>5,15</sup> Intra-laboratory variables that may affect test results include pH of the purified water used for reagent reconstitution, pipetting technique, incubation time and temperature, reagent contamination, or change in reagent lot number.<sup>18,19</sup> Periodic quality control tests, performed on a regular basis, will help to identify any variations that may occur and lead to erroneous test results.

#### RESULTS

UPTT test results should be reported as the clotting endpoint in seconds. Tests and controls shall be run in triplicate and results averaged prior to comparing them to the laboratory's established reference range.<sup>14,16,17,22</sup>

A p-value should be included with the results as an indicator of test validity.

Statistically compare the averaged results from the unexposed plasma to the results from the plasma exposed to the device. To pass, the device statistic must have a p-value of  $\geq 0.05$ .

#### EXPECTED VALUES

Normal Plasma: >60 seconds  
 Level 1: >60 seconds  
 Level 2: > 90 seconds  
 Level 3: >120 seconds

**NOTE: UPTT results, when tested with normal control plasma on a photo optical analyzer, will be greater than 60 seconds. Contact activation will shorten this time. Because of the operational variability among analyzers and protocols, each laboratory must establish a reference range for the UPTT. The experimental protocol, sample preparation and endpoint detection method may influence test results.**

#### LIMITATIONS

1. Hemolyzed, icteric or lipemic plasma may produce erroneous test results.
  - a. The concentration of hemolysis, icterus or lipemia that will affect test results is analyzer dependent.<sup>17,21</sup>
2. Freezing plasma samples may cause erroneous test results.
3. Incorrect ratio of blood to anti-coagulant may cause spurious test results. The quantity of anticoagulant added to blood must be proportionally decreased in specimens with hematocrit values above 53%, and increased for those with hematocrit values below 25%.<sup>2,7</sup>

- The use of coagulation analyzers whose detection system is mechanical or combined optical-mechanical is **not** recommended. Mechanical detection of the clotting endpoint will contribute to contact activation, and report undetected or significantly shortened test times.

#### PERFORMANCE CHARACTERISTICS

UPTT Reagent has been tested with manual and photo-optical detection systems. The precision and sensitivity are sufficient to provide reliable results for hemocompatibility and thrombogenicity tests based on contact activation.<sup>3,4,6,20</sup>

#### References

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