

**NSF ITR Project  
Fall 2004**

**General Notes on Trypsin Test Procedure:**

Automated performance procedure:

- Label plates (using bar code)
- Prepare sample dilution:
  - o Use stock solution in deep well plate to create sample plates (with single-channel pipeting tool).
  - o Create flat (sample) plates with different concentrations of samples (from deep well plate).
  - o Prepare test plates:
    - Transfer dilutions from sample plates to test plates.
    - Combine dilutions with reagents (contained in reservoirs at pipeting stations - Biomek 2000 or Biomek FX):
      - o Buffer
      - o Trypsin solution (control)
      - o Solutions containing no Trypsin (CaCL) (control blank)
      - o BPNA (enzyme substrate or reagent)
      - o Acetic acid (reagent)

(Note: See pipeting steps on copy of poster of Trypsin assay.)

- o Incubation step:
  - Preheat incubator to 37°C (normal body temperature. (This is optimal temperature for Trypsin reaction. Trypsin breakdowns substrate, BAPNA – enzyme substrate, at this temperature.).
  - Insert plates in incubator (manually) using backdoor of device.
  - Incubation for preset duration.
- o Plate reader – measures absorbance:
  - Specify wavelength of light for measurement (“yellow”).
  - Get raw data – absorbance levels for each column in plate (control, control blank, sample, sample blank).
  - Conduct basic statistical analysis to identify outliers (depends on sample size and sample values) → Dixon test
  - Calculations using Excel:
    - Average across replicates
    - Standard deviation
    - Relative standard deviation (CV[%])
    - Control - control blank and sample – sample blank respectively (preliminary calculation).
      - o Calculate IC50 for standard compound (create curve from 8 data points)
    - Normalized trypsin activity [%] (= activity level)

- Graphical display – create graph of activity levels for each compound
  - Compare activity level with criteria activity.
  - Decide whether compound is active.
  - For standard compound: Determination of IC50 by Statistic program “Origin” (curve fitting) to compare with former results (“Does the assay still work”?)
  - Calculation of the Z’ – Factor (quality criteria)

Manual performance procedure:

(Uses/purpose of procedure:

Setup a new assay.

Use as basis for comparison with automated version of assay (make sure results are good).

Use of a standard compound in manual process.

Helps operators understand technology of assay (basis for programming SAMI system).

- Label vials and plates with pen.
- Create sample dilutions in tubes - use vials.
  - A master vial is used to create sample vials (single-channel tool).
- Prepare test plates – use flat plates, vials, and reservoirs (using single-channel and eight channel pipet tools)
  - Test plates are created by adding dilutions and reagents to wells following same procedure as used in automated version of process.)
- Incubation step:
  - Human handling of plates in same manner as robotic handling.
- Plate reader use:
  - Occurs in same manner as use in automated process.
  - Operator looks at data through plate reader software.  
(Excel-based package allowing for viewing in plate format or text file.)
  - Conduct basic statistical analysis to identify outliers (depends on sample size and sample values) → Dixon test
  - Calculations using Excel:
    - Average across replicates
    - Standard deviation
    - Relative standard deviation (CV[%])
    - Control - control blank and sample – sample blank respectively  
(preliminary calculation)
    - Normalized trypsin activity [%] (= activity level)
    - Graphical display
  - Determination of IC50 by Statistic program “Origin” (curve fitting)
  - Compare of activity levels (reproducibility)
  - Comparison of IC50 values of manual procedure and automated procedure