

Test Item Information Sheet (TIIS)

“Cell Viability” Scheme [CELL24]

This sheet contains all the information on **Viable Cells Test Items** that you should be aware of to conduct the above mentioned Scheme. **Please read carefully before performing any operation and/or test on the provided samples.**

Test Items Description

- Source material: Jurkat cell line.
- Method of preparation: Culture in RPMI1640 10% FBS HI (Heat Inactivated + antibiotics) at a concentration of 5×10^6 to 1×10^7 cells/mL in T175 cm² in a humidified incubator at 37°C, 5% CO₂.
- Concentration: Approximately 5×10^6 cells/mL (total volume of 1mL) per vial.
- Medium: Jurkat cells are frozen in an animal protein-free, serum-free and defined cryopreservation medium containing 10% dimethyl sulfoxide (DMSO) (CRYOSTOR CS10).
- Date of preparation and any lot number (if applicable): June - August 2024.
- Biological hazard: The source material is BSL 1.
- Biosafety level: All operations have been conducted in a BSL 2 environment.
- Method used for value assignment: Consensus mean from Participants.
- Homogeneity and Stability information: Homogeneity and stability of the Test Items will be controlled August 2024 to be compliant with the requirements of *The International harmonized protocol for the proficiency testing of analytical chemistry laboratories*, IUPAC technical report.

Instructions to Prepare the Test Items for Testing

- Processing required of Test Item: No processing is required at receipt of Test Item.
- Any thawing/freezing and/or other processes at receipt: Put in liquid nitrogen (or LN vapour).
- Any storage requirement between receipt and testing date: Store in liquid nitrogen (or LN vapour). Testing should be performed within 1 week of receipt.
- Required temperature to perform the testing: Room temperature (18-24°C).
- Any step required/recommended for testing:
 - *Thawing method*: Rapid thaw in 37°C waterbath or equivalent;
 - *Time to start of testing*: As soon as possible (immediately after thawing, i.e. within 10-15min);
 - *Dilution factor*: 1:10 dilution in cell culture media after thaw;
 - *Conditions prior to testing*: Maintain at room temperature (18-24°C) until testing.
- Any factor that may impact the testing negatively: Slow thawing; contamination of Test Item; harsh centrifugation conditions; Test Items kept in ice; too long time between thawing and processing; inefficient removal of cryopreservation medium.

Particular Handling/Safety Requirements

- Potential risks of Test Item: The DMSO contained in the freezing medium is an irritant that readily penetrates the skin. Residual liquid nitrogen in the micro-tubes may present an explosive hazard.
- Individual protection equipment required: Blouse, mask, glasses and gloves.
- In case of puncture or cuts: Wash thoroughly with water and then disinfect during 10 minutes. Contact a doctor. Make a statement of incident in the laboratory’s registry, the laboratory’s administration and the laboratory’s medical service.



- In case of contact with the eye: Wash thoroughly with water or physiologic serum during 5 minutes; contact an ophthalmologist or a doctor; same procedure of declaration than previously.
- In case of contact with mucus membranes and skin: Wash thoroughly with water and contact a doctor.
- Measures to take in case of accidental spillage: Cover with bleach. Clear the zone and let act 30 minutes. Eliminate waste with absorbing paper. Use disinfectant and thoroughly clean the effected surface.
- Waste elimination procedures: Waste generated by healthcare activities, to eliminate in incinerable plastic containers, correctly identified and marked with the “biohazard” pictogramme.

Schemes Specifications

- For each Test Item (Tube A, Tube B and Tube C):
 - Manual counting: Please indicate the **percentage of viable cells**.
 - Image-based cell counting: Please indicate the **percentage of viable cells**.
 - Flow Cytometry: Please indicate the **percentage of viable cells**. If your method allows, please also indicate percentage of **early apoptotic cells**.
- How to test your samples: please test the Test Items following your usual routine testing method(s). We recommend that you exclude debris from your count.
- You will be asked to report your results under the following methods: **Manual Counting, Image-based Cell Counting, Flow Cytometry**.
- Please be ready to enter the following additional information while reporting your results:
 - Manual Counting: type of equipment, thawing method, dilution used, how many squares were measured, timing of measurement;
 - Image-based Cell Counting: type of equipment, thawing method, dilution used, timing of measurement;
 - Flow cytometry: type of equipment, type of Markers/Fluorochromes, Excitation wavelengths and Emission filters, thawing method, dilution used, timing of experiment;
 - Equipment performance verification: please enter the frequency of verification runs and the last verification date and results.

What and How to Submit

- For each Test Item, you can perform the assay more than once per method (according to your selected routine method), and submit more than one test result.
- Your results must be submitted online to the PT website <http://biospecimenpt.ibbl.lu/> using the login information (Laboratory Number and Password) provided to you via email after the registration to the “Cell Viability” Scheme.
- Please complete the questionnaire as accurately as possible, adding any relevant detail and comment in the appropriate comment section.

Timelines

<i>Results submission</i>	<i>Data analysis & Report preparation</i>	<i>Reports available</i>
17 NOV 2024, latest	20 NOV 2024– 31 JAN 2025	March 2025

In case of doubts in the completion phase, please contact IBBL at ISBERPT@ibbl.lu