Test Item Information Sheet (TIIS)

"Cell Viability" 2020_R1 Scheme

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 x 10^6 to 1 x 10^7 cells/mL in T175 cm^2 in a humidified incubator at 37°C, 5% CO_2.
- Concentration: Approximately 5 x 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 2018, June 2019.
- 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 were controlled in June 2018 and June 2019 and were found 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;
  - *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: Abundantly wash 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 projection in the eye: Abundantly wash with water or physiologic serum during 5 minutes; contact an ophthalmologist or a doctor; same procedure of declaration than previously.
- In case of projection on the mucous membranes and skin: Wash with water and contact a doctor.
- Measures to take in case of accidental dispersion: Cover with bleach. Clear the zone and let act 30 minutes. Eliminate waste with absorbing paper. Pulverise disinfectant and clean the concerned surface completely.
- 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):
  - Trypan Blue Staining method: Please indicate the **percentage of viable cells.**
  - Flow Cytometry method: 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: **Trypan Blue Staining**, **Flow Cytometry**.
- Please be ready to enter the following additional information while reporting your results:
  - Trypan Blue staining: type of equipment, thawing method, dilution used, how many squares were measured, time of thawing, time at which you started your procedures (i.e. staining), time at which you started your measurement acquisition;
  - Flow cytometry: type of equipment, type of Markers/Fluorochromes, Excitation wavelengths and Emission filters, thawing method, dilution used, time of thawing, time at which you started your procedures (i.e. staining), time at which you started your measurement acquisition;
  - 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

Results submission	*Data analysis & Report preparation*	*Reports available*
20 NOV 2020, **latest**	23 NOV 2020– 22 JAN 2021	26 FEB 2021

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