Xpert® BCR-ABL Ultra p190 (CE-IVD)

For use in the UK NEQAS for Leucocyte Immunophenotyping Minor BCR-ABL1 Quantification Program

Disclaimer: This protocol was developed by Cepheid Oncology Research and Development and Medical and Scientific Affairs staff and describes an alternate sample preparation process for proficiency testing (PT) / external quality assurance (EQA) samples tested with the Xpert® BCR-ABL Ultra p190 test. This document is provided as a courtesy to Cepheid customers to provide guidance for participation in the UK NEQAS LI Minor BCR-ABL1 Quantification Program. Cepheid does not endorse the testing of alternate specimen types (i.e., specimen types not included in the product labeling) with registered (FDA, CE, or other registration body) tests without proper validation, but recognizes the medical need of such testing for proficiency testing. If you choose to use Xpert® BCR-ABL Ultra p190 with alternate specimen types, it is your laboratory's responsibility to validate the assay in accordance with federal, state/ province, and local laws. Additional testing of quality control samples and verification of other performance characteristics of the test also may need to be completed.

Objective

The objective of this document is to provide a protocol for the use of samples provided by UK NEQAS for Leucocyte Immunophenotyping (LI) in the Minor BCR-ABL1 Quantification program using the Xpert® BCR-ABL Ultra p190 test. **Please read the full protocol and the additional tips at the end of the protocol before you start the testing.**

Materials

- 1 UK NEQAS LI samples (glass vials) – containing lyophilized cell line material yields sufficient final lysate for 20 replicates
  - 9 x 10^6 cells per vial = ~0.335 million cells in 4.5ml final lysate going into the Xpert® BCR-ABL Ultra p190 cartridge
- Xpert® BCR ABL Ultra p190 Cartridges (part number: GXBCRABLP190-CE-10)
- Lysis Reagent (provided in the Xpert BCR-ABL Ultra p190 kits)
- Proteinase K (provided in the Xpert BCR-ABL Ultra p190 kits)
- DNA/RNA–free water (not provided)
- Reagent grade absolute ethanol (not provided)
- Vortex mixer (not provided)
- Pipettes and aerosol filter pipette tips (not provided)
- 50 mL conical tubes (not provided)

Protocol

- Bring the Xpert® BCR-ABL Ultra p190 cartridges/reagents to room temperature by removing from refrigerator at least 20 minutes prior to use.

- Definitions:
  - Vortexing = High speed
  - Swirling = 10 times in 5 seconds

1. Incubate the UK NEQAS LI samples (glass vial[s]) for testing at room temperature for 5 minutes.

2. For each UK NEQAS LI sample vial, label two 50 mL conical tubes with the vial ID and then mark one as tube (1) and the other as tube (2).

Note: Follow steps 3-18 for each sample. Once Lysis Reagent is added to the lyophilized cells, the sample needs to be processed right away. Preferably run samples one at a time in order to be able to adhere to stated incubation times.

3. Before opening, tap the vial gently to collect the lyophilized material at the bottom of the vial.

4. Add 1.5 mL Lysis Reagent to the lyophilized cells in the glass vial.

Note: The rubber stopper on top of the glass vial can have lyophilized material sticking to it. Therefore, after adding lysis reagent to the glass vial, recap the rubber stopper and make sure lysis reagent reaches all lyophilized material including anything sticking to the rubber lid.

5. Gently swirl to encompass all cells and leave for four minutes at room temperature. Repeat this step three times to ensure cell pellet is fully hydrated.

6. Add 100µL Proteinase K (PK) to conical tube (1).

7. After adding lysis reagent to the glass vial, set pipette to 0.8 mL (800 µL). Ensuring the end of the pipette tip is positioned at the bottom of the glass vial, gently pipette 20 times to mix and break down cell pellet. Pipette gently to avoid bubbles forming. While pipetting, move the tip around over the bottom of the vial to ensure that the entire pellet is being disrupted. Transfer the cell resuspension to conical tube (1).

Note: Label on the glass vial can obstruct the view of cells sticking to the wall. Make sure to get the cells from all sides.

8. Add an additional 1.0 mL Lysis Reagent to the glass vial. Using a 1 mL pipette, repeatedly and gently pipet the Lysis Reagent to wash any cellular material from the walls/bottom of the vial before transferring it into conical tube (1).

9. Add 1.0 mL DNA/ RNAse-free water to the glass vial to wash the walls and transfer into conical tube (1).

Note: The most crucial steps are the reconstitution of the cell pellet (steps 5, 7, and 8) to ensure that all of the cell material from the glass vial is transferred to the conical tube (1) for further processing. Good light conditions are required in order to discover any undissolved cells sticking to the walls or bottom of the vial. It is advisable to inspect vial before and after step 8 and 9. Transfer all material, including any bubbles.

10. Add additional 3.0 mL DNA/ RNAse-free water to the conical tube (1).

Note: It is important to follow the specified vortexing times in steps 11, 14, and 15.

11. Swirl conical tube (1) before vortexing for 30 seconds.

12. Incubate at RT for 20 minutes to allow the cells to lyse. During this incubation perform the following steps:
    a. Add 0.45 mL DNA/ RNAse-free water to a new conical tube (2).
    b. Add 1.80 mL Lysis Reagent to conical tube (2).

13. Swirl the cell lysate in conical tube (1) before transferring 0.25 mL cell lysate to conical tube (2).

Note: There might be a lot of bubbles/foam after vortexing tube (1) (step 11). Ensure that you actually aspirate 0.25 mL of liquid and not any bubbles/foam.

14. Vortex conical tube (2) for 20 seconds.

15. Add 2 mL ethanol to conical tube (2) and vortex for 30 seconds.

16. Add the entire sample to the Xpert® BCR-ABL Ultra p190 cartridge.

17. Add the Wash Reagent and load the cartridge on to the GeneXpert® System (as described in the package insert).[1,2]

18. Retain the remaining lysate from conical tube (1) for retesting if required (following steps 13 -16). Store the lysate at -80 ºC. Before retesting, thaw out the retained lysate completely to room temperature and vortex for 10 seconds before use. If repeats are not required, discard extra lysate.

19. Analyse data generated with Xpert® BCR-ABL Ultra p190 cartridges per your usual method.

Note: The most crucial step of this process is the full reconstitution of lyophilized cells and complete transfer of all material to the conical tube.

- The lyophilized material in each vial can differ slightly and the ease/difficulty when pipetting to break down the pellet may vary.
- While pipetting, move the tip around over the bottom of the vial to ensure that the entire pellet is being disrupted.
- Look closely at vials in proper light conditions to check for any remaining cells sticking to the vial wall and bottom.
- Check the vials carefully when first adding lysis reagent and then water to the glass vial in order to not miss any remaining cell material.
- Repeatedly and gently pipette first the lysis reagent and then the water during those two washing steps.
- Make sure the entire wall and bottom surface is being washed.
- The reconstituted material needs to be processed right away. Do NOT store the cells dissolved in lysis buffer overnight before running the assay.

Questions

For any questions or additional information on the proficiency testing, please contact NEQAS:
UK NEQAS for Leuocyte Immunophenotyping
Pegasus House, 4th Floor Suite, 463A Glossop Road
Sheffield, S10 2QD, United Kingdom
www.ukneqasli.co.uk

For any questions or additional information related to this guideline, please contact:
Medical and Scientific Affairs (Oncology) at MedSci.Oncology@cepheid.com.

For general technical support questions, please contact:
Technical Support at techsupport@cepheid.com or support@cepheideurope.com

REFERENCES

1 Xpert® BCR-ABL Ultra p190 Package Insert. Sunnyvale. USA. 2022. (CE-IVD). 302-0742

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