

## **Xpert® NPM1 Mutation (CE-IVD)\***

# **Technical Guideline for using Xpert NPM1 Mutation\* test in the UK NEQAS for Leucocyte Immunophenotyping NPM1 Mutation Status 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 NEQAS proficiency testing (PT) / external quality assurance (EQA) samples tested with the Xpert NPM1 Mutation test. This document is provided as a courtesy to Cepheid customers to provide guidance for participation in the UK NEQAS NPM1 Mutation Status 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 NPM1 Mutation test with alternate specimen types, it is your laboratory's responsibility to validate the assay in accordance with all applicable federal, state/province, and local laws. Additional testing of quality control samples and verification of other performance characteristics of the test may also 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 NPM1 Mutation Status Program using the Xpert NPM1 Mutation test. Please read the full protocol and the additional tips at the end of the protocol before you start the testing.

### **Materials**

- 2 UK NEQAS LI samples (glass vials) — containing lyophilised cell line material
  - 7 x 10<sup>6</sup> cells per vial = 0.39 million cells in 4.5 ml final lysate going into the Xpert NPM1 Mutation cartridge

The proficiency testing provider of the samples is: UK NEQAS for Leucocyte Immunophenotyping, Pegasus House, 4th Floor Suite, 463A Glossop Road, Sheffield, S10 2QD, United Kingdom. [www.ukneqasli.co.uk](http://www.ukneqasli.co.uk)
- 2 Xpert NPM1 Mutation cartridges (part number: GXNPM1-CE-10)
- Lysis Reagent (provided in the Xpert NPM1 Mutation kits)
- Proteinase K (provided in the Xpert NPM1 Mutation 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 NPM1 Mutation 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 Falcon 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.**

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

**4** Add **1.5 mL Lysis Reagent** to the lyophilised 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 the 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 reconstitution.**

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

**7** After adding lysis reagent to the glass vial, set pipette to 0.8mL (800µL). 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 **Falcon 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 **Falcon tube (1)**.

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

**NOTE:** The most crucial steps are the reconstitution of the cell pellet (steps 5, 7, and 8) to ensure that all the cell material from the glass vial is transferred to the **Falcon 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 **Falcon tube (1)**.

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

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

**12** Incubate at room temperature for 20 minutes to allow the cells to lyse. During this incubation perform the following steps:

a. Add **0.51 mL DNA/RNase-free water** to a new **Falcon tube (2)**

b. Add **1.60 mL Lysis Reagent** to **Falcon tube (2)**

**13** Swirl the **cell lysate in Falcon tube (1)** before transferring **0.375 mL cell lysate** to **Falcon tube (2)**.

**NOTE:** There might be a lot of bubbles/foam after vortexing **Falcon tube (1)** (step 11). Ensure that you actually aspirate 0.375 mL of liquid and not any bubbles/foam. Vortex **Falcon tube (2)** for 20 seconds.

**14** Vortex **Falcon tube (2)** for 20 seconds.

**15** Add **2 mL Ethanol** to **Falcon tube (2)** and vortex for 30 seconds.

**16** Add the entire sample to the Xpert NPM1 Mutation **cartridge**.

- 17** Add the **wash reagent** and load the cartridge onto the GeneXpert® system (as described in the package insert).
- 18** Retain the remaining lysate from **Falcon tube (1)** for retesting if required (following steps 13–16). Store the lysate at -80 °C. 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** Repeat the above process for the second sample vial.
- 20 Analyse data generated with Xpert NPM1 Mutation cartridges per your usual method.**

**NOTE:** The most crucial step of this process is the full reconstitution of lyophilized cells and the complete transfer of all material to the Falcon 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 are 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

**The proficiency testing provider of the samples is:**

UK NEQAS for Leucocyte Immunophenotyping  
Pegasus House, 4th Floor Suite, 463A Glossop Road  
Sheffield, S10 2QD, United Kingdom  
[www.ukneqasli.co.uk](http://www.ukneqasli.co.uk)

Contact NEQAS for questions regarding the EQA samples.

**For other questions or additional information,** please contact Technical Support at [techsupport@cepheid.com](mailto:techsupport@cepheid.com), [support@cepheideurope.com](mailto:support@cepheideurope.com), or Medical/Scientific Affairs (Oncology) at [MedSci.Oncology@cepheid.com](mailto:MedSci.Oncology@cepheid.com).

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