LymphoTrack® Assays for the Ion PGM™, Workflow

Interest in any of the products, request or order them at Bio-Connect Diagnostics.
These products are sold for Research Use Only; not for use in diagnostic procedures.

**LymphoTrack® Assays**

1. **Prepare DNA samples**
2. **Run PCR with a LymphoTrack Assay**
3. **Purify amplicons**
4. **Quantify & prepare library**
5. **Load & run Ion PGM**
6. **Process data with included bioinformatics**
7. **Analyze**

**Unparalleled workflow simplicity**

LymphoTrack Assays put Ig/TCR next-generation sequencing at your fingertips.

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**Workflow Summary**

01. Using gloved hands, remove the Master Mixes from the freezer. Allow the tubes to thaw; then gently vortex to mix.

02. In a containment hood or dead air box, pipette 45 μL of Master Mix into individual wells of a PCR plate (a different indexed Master Mix should be used for each sample and control).

03. Add 0.2 μL EagleTaq™ DNA polymerase to each Master Mix.

04. Add 5 μL of DNA from the unknown samples and controls to wells containing the respective Master Mix reactions and pipette up and down 5-10 times to mix.

05. Add 5 μL of molecular biology grade water to the well containing the Master Mix for the no template control and pipette up and down 5-10 times to mix.

06. Seal the plate and amplify target DNA using the following thermocycler program:

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95 °C</td>
<td>7 minutes</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>95 °C</td>
<td>45 seconds</td>
<td>29x</td>
</tr>
<tr>
<td>3</td>
<td>60 °C</td>
<td>45 seconds</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>72 °C</td>
<td>90 seconds</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>72 °C</td>
<td>10 minutes</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>15 °C</td>
<td>‘Forever’</td>
<td>1</td>
</tr>
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</table>

07. Remove the amplification plate from the thermocycler.

08. Purify the PCR products using the Agencourt® AMPure® XP PCR Purification system. Add 90 μL of particles to each 50 μL reaction; elute DNA in 40 μL of eluant.

09. Quantify amplicons with an appropriate method (e.g. Bioanalyzer® 2100 or LabChip® GX).

10. Create the library by combining 4 nM of each amplicon in a tube (do not include the no template control).

11. Dilute the library.

12. Using the OT2 instrument and the Ion PGM Template OT2 400 Kit or Ion PGM Hi-Q OT2 Kit (or OT2 200 Kit if only testing TRG), perform an emulsion PCR to create template-positive ion sphere particles (ISPs).

13. Enrich the template-positive ISPs with the Ion OneTouch™ ES.

14. Initialize the Ion PGM and load the 316 v2 chip with ISPs.

15. Create a Planned Run using the Torrent Browser.

16. Start the Ion PGM run.

17. Analyze and visualize the acquired data using the LymphoTrack Software for the Ion PGM.

**Storage Conditions:** -65 °C to -85 °C (DNA controls may be separated from kits and stored at 2 °C to 8 °C).

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**Ordering Information**

<table>
<thead>
<tr>
<th>CATALOG #</th>
<th>PRODUCTS</th>
<th>QUANTITY</th>
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<tbody>
<tr>
<td>7-121-0057</td>
<td>LymphoTrack® IGH FRI/2/3 Assay – PGM™</td>
<td>12 indices - 5 sequencing reactions each</td>
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<tr>
<td>7-121-0007</td>
<td>LymphoTrack® IGH FRI Assay – PGM™</td>
<td>12 indices - 5 sequencing reactions each</td>
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<td>LymphoTrack® IGH FR2 Assay – PGM™</td>
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<td>7-227-0007</td>
<td>LymphoTrack® TRG Assay – PGM™</td>
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<tr>
<td>7-500-0007</td>
<td>LymphoTrack® Software – PGM™</td>
<td>1 CD complimentary with purchase</td>
</tr>
<tr>
<td>7-500-0008</td>
<td>LymphoTrack® MRD Software</td>
<td>1 CD complimentary with purchase</td>
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