

CytoScan™ Accel Assay Manual Workflow, 8 Samples

Pub. No. MAN0028087 Rev. A.0

IMPORTANT! This quick reference is intended for users who are familiar with the CytoScan™ Accel Assay Manual Workflow and plan to perform the assay without stopping.

This quick reference differs from the user guide. It does not contain as much detail, the optional stopping points, and the assay steps are in a different order. Users must be familiar with the assay and the user guide before using this quick reference.

Note: For safety and biohazard guidelines, see the “Safety” appendix in the following product documentation: *CytoScan™ Accel Assay Manual Workflow User Guide* (Pub. No. MAN0028085). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

General procedures

Laboratory

- Set up the laboratory areas for a single-direction workflow from Pre-PCR to Post-PCR.
- Store reagents in the appropriate Pre-PCR or Post-PCR Room as detailed on the packaging.
- Never bring amplified products into the Pre-PCR Room.
- Do not mix and match reagents from other reagent kits.
- Properly chill equipment such as cooling blocks and reagent coolers before use.
- Unless otherwise indicated, keep all reagents on ice, or on a chilled cooling block on ice.
- Ensure that the enzymes are kept at -25°C to -15°C until needed. When removed from the freezer, immediately place in a benchtop reagent cooler that has been chilled to -25°C to -15°C .

Vortexing

- Always ensure that the plates are tightly sealed. A tight seal prevents sample loss and cross-well contamination, particularly when plates are being vortexed.
- Reagent vials: Vortex 3 times, 1 second each time.
- Enzyme vials: Vortex for 1 second, 1 time.
- Master mix tubes: Vortex 3 times, 1 second each time. Do this before and after adding an enzyme.
- Vortex plates: Vortex 3 seconds in all corners and in the center.
- When instructed, *repeat* the vortex process for high-volume reactions: PCR (100.0 μL), and hybridization-ready samples (260.5 μL).

Centrifuging




- Reagent vials: Centrifuge for 3 seconds using a benchtop mini centrifuge.
- Enzyme vials: Centrifuge for 3 seconds using a benchtop mini centrifuge.
- Master mix tubes: Centrifuge for 3 seconds using a benchtop mini centrifuge.
- Strip tubes: Centrifuge for 3 seconds using a benchtop mini centrifuge.
- Plates: Centrifuge at room temperature, 1 minute at 650 x *g* using a plate centrifuge.
- During Stage 7: Fragmentation, centrifuge the **Fragmentation Plate** at 4°C in a refrigerated centrifuge.

Stage 1: Digestion

Perform the following steps in the Pre-PCR Room.

1. Prepare the Pre-PCR Room:
 - a. Power on the thermal cycler in the Pre-PCR room.
 - b. Fill the ice bucket with ice.
 - c. Place the cooling block on ice.
2. Prepare the **Sample Plate**:
 - a. If frozen, thaw the **Sample Plate** (5 μL of 20 ng/ μL = 100 ng) at room temperature.
 - b. Vortex, centrifuge, then place it on the lower half of the cooling block.
3. Place an 8-well strip tube on the cooling block. Label a 1.5-mL tube "Dig", then place it on the cooling block.
4. Prepare the Digestion reagents:
 - a. Place the Nuclease-Free Water on ice.
 - b. Thaw the Xce I Buffer at room temperature.
 - c. Once thawed, place it on ice.
5. Prepare the Xce I Buffer. Vortex, centrifuge, then place it on the cooling block.
6. Prepare the Digestion Master Mix as shown in the following table. Add Nuclease-Free Water and Xce I Buffer to the 1.5-mL tube labeled "Dig". Vortex, centrifuge, then place it on the cooling block.

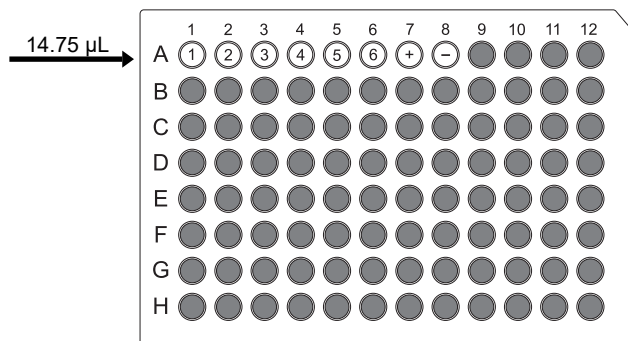
Table 1 Digestion Master Mix (≥ 8 samples, 20% overage).

Reagent	1 sample	8 samples
 Nuclease-Free Water	11.75 μL	112.8 μL
 Xce I Buffer	2.00 μL	19.2 μL
 Xce I	1.00 μL	9.6 μL
Total	14.75 μL	141.6 μL

7. Remove the Xce I enzyme from the freezer and immediately place it in a cooler. Centrifuge for 1 second, then vortex 1 second. Centrifuge for 3 seconds, then place it in the cooler.
8. Immediately add the Xce I enzyme to the Digestion Master Mix tube, then return the Xce I enzyme to the cooler.
9. Vortex and centrifuge the Digestion Master Mix, then place it on the cooling block.
10. Aliquot the Digestion Master Mix into the strip tube. Add 17 μL into wells 1–8. Place the strip tube on the cooling block.
11. Remove the seal from the **Sample Plate**.

12. Use a multichannel P20 pipette to add 14.75 μL of the Digestion Master Mix to each sample in the plate.

Samples	Volume/sample
Genomic DNA (20 ng/ μL)	5.0 μL
Digestion Master Mix	14.75 μL
Total volume	19.75 μL



The plate is now called the **Digestion Plate**.

13. Seal the **Digestion Plate**, vortex, then centrifuge.
14. Load the **Digestion Plate** in the thermal cycler and run the CytoScan Accel Digest protocol.

Temperature	Time
37°C	10 minutes
65°C	20 minutes
4°C	∞

15. While the CytoScan Accel Digest protocol is running, prepare the Ligation reagents:
 - a. Thaw the T4 DNA Ligase Buffer and Adaptor Nsp I at room temperature.
 - b. Once thawed, place them on ice.
16. Prepare the PCR reagents:
 - a. Place the Nuclease-Free Water and GC-Melt Reagent on ice.
 - b. Thaw the 10X TITANIUM™ Taq PCR Buffer, dNTP Mixture and PCR Primer at room temperature.
 - c. Once thawed, place them on ice.

Note: Ligation reagents and PCR reagents are vortexed and centrifuged in later steps.
17. When CytoScan Accel Digest protocol is complete, take the **Digestion Plate** out of the thermal cycler and place it on the cooling block. Centrifuge the plate, then place it on the lower half of the cooling block.
18. Immediately start "Stage 2: Ligation" on page 3.

Stage 2: Ligation

Perform the following steps in the Pre-PCR Room.

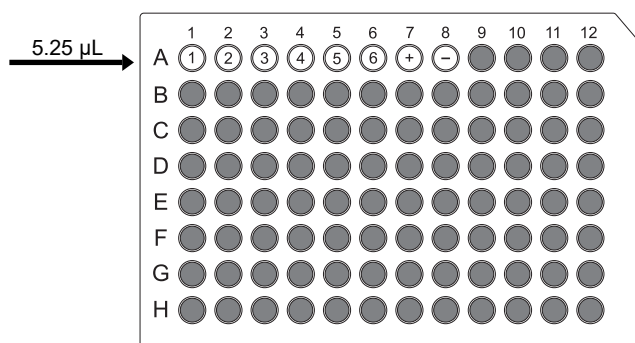
1. Place an 8-well strip tube on the cooling block. Label a 1.5-mL tube “Lig”, then place it on the cooling block.
2. Prepare the T4 DNA Ligase Buffer and the Adaptor Nsp I. Vortex, centrifuge, then place them on the cooling block.
Note: If precipitate is present in T4 DNA Ligase Buffer, allow it to warm to room temperature, then vortex until precipitate is dissolved.
3. Prepare the Ligation Master Mix as shown in the following table. Add T4 DNA Ligase Buffer and Adaptor Nsp I to the 1.5-mL tube labeled “Lig”. Vortex, centrifuge, then place it on the cooling block.

Table 2 Ligation Master Mix (≥8 samples, 25% overage).

Reagent	1 sample	8 samples
T4 DNA Ligase Buffer	2.5 µL	25.0 µL
Adaptor Nsp I	0.75 µL	7.5 µL
T4 DNA Ligase	2.00 µL	20.0 µL
Total	5.25 µL	52.5 µL

4. Remove the T4 DNA Ligase from the freezer and immediately place it in a cooler. Centrifuge for 1 second, then vortex 1 second. Centrifuge for 3 seconds, then place it in the cooler.
5. Immediately add the T4 DNA Ligase to the Ligation Master Mix tube, then return the T4 DNA Ligase to the cooler.
6. Vortex and centrifuge the Ligation Master Mix, then place it on the cooling block.
7. Aliquot the Ligation Master Mix into the strip tube. Add 6 µL into wells 1—8. Place the strip tube on the cooling block.
8. Remove the seal from the **Digestion Plate**.
9. Use a multichannel P20 pipette to add 5.25 µL of the Ligation Master Mix to each sample in the plate.

Samples	Volume/sample
Digested DNA	19.75 µL
Ligation Master Mix	5.25 µL
Total volume	25.00 µL



The plate is now called the **Ligation Plate**.

10. Seal the **Ligation Plate**, vortex, then centrifuge.
 11. Load the **Ligation Plate** in the thermal cycler and run the CytoScan Accel Ligate protocol.
- | Temperature | Time |
|-------------|------------|
| 16°C | 10 minutes |
| 70°C | 20 minutes |
| 4°C | ∞ |
12. When CytoScan Accel Ligate protocol is complete, take the **Ligation Plate** out of the thermal cycler and place it on the cooling block. Centrifuge the plate, then place it on the lower half of the cooling block.
 13. Immediately start “Stage 3: PCR” on page 4.

Stage 3: PCR

The PCR is performed in 2 rooms:

- Perform PCR preparation in the Pre-PCR Room.
- Perform the CytoScan Accel PCR thermal cycler protocol in the Post-PCR Room.

1. Prepare the Post-PCR Room: Power on the thermal cycler in the Post-PCR room.
2. Prepare the Pre-PCR Room:
 - a. Refresh ice in the ice bucket.
 - b. Place the cooling block on ice.
 - c. Place a reagent reservoir on the upper half of the cooling block.
3. Pour 3 mL of Nuclease-Free Water into the reagent reservoir.
4. Remove the seal from the **Ligation Plate**.
5. Use a multichannel P200 pipette to add 75 μ L of Nuclease-Free Water to each sample in the plate.

Samples	Volume/sample
Ligated DNA	25.0 μ L
Nuclease-Free Water	75.0 μ L
Total volume	100.0 μL

The plate is now called the **Diluted Ligation Plate**.

6. Seal the **Diluted Ligation Plate**, vortex, then centrifuge.
 7. Place the **Diluted Ligation Plate** on the upper half of the cooling block.
 8. Label a 96-well plate "PCR", then place it on the lower half of the cooling block.
- This plate is called the **PCR Plate**.
9. Remove the seal from the **Diluted Ligation Plate**.
 10. Use a multichannel P20 pipette to transfer 10 μ L of each sample from the **Diluted Ligation Plate** to the corresponding 4 wells of the **PCR Plate**.

Note: Each sample will have 4 PCR reactions.

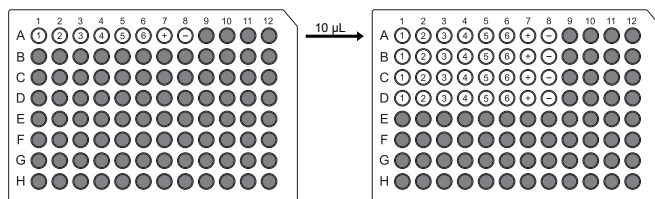


Figure 1 Diluted Ligation Plate to PCR Plate.

11. Seal the **PCR Plate**, then place it on the lower half of the cooling block.
12. Seal the **Diluted Ligation Plate**, then store at -25°C to -15°C .

13. Label a 15-mL tube "PCR MM", then place it on ice.
14. Place a reagent reservoir on the upper half of the cooling block.
15. Prepare the 10X TITANIUM™ Taq PCR Buffer, GC-Melt Reagent, and dNTP Mixture. Vortex, then place them on ice.
16. Prepare the PCR Primer. Vortex, centrifuge, then place it on the cooling block.
17. Prepare PCR Master Mix as shown in the following table. To the 15-mL tube labeled "PCR MM", add all the reagents except the 50X TITANIUM™ Taq DNA Polymerase Mix. Vortex *continuously* for 3 seconds, then place it on ice.

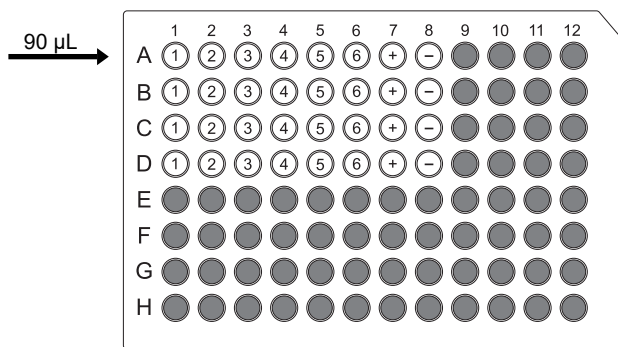
Table 3 PCR Master Mix (≥ 8 samples, 15% overage).

Reagent	1 reaction	8 samples
Nuclease-Free Water	39.5 μ L	1,453.6 μ L
10X TITANIUM™ Taq PCR Buffer	10.0 μ L	368.0 μ L
GC-Melt Reagent	20.0 μ L	736.0 μ L
dNTP Mixture	14.0 μ L	515.2 μ L
PCR Primer	4.5 μ L	165.6 μ L
50X TITANIUM™ Taq DNA Polymerase Mix	2.0 μ L	73.6 μ L
Total	90.0 μL	3,312.0 μL

18. Remove the 50X TITANIUM™ Taq DNA Polymerase Mix from the freezer and immediately place it in a cooler. Centrifuge for 1 second, then vortex 1 second. Centrifuge for 3 seconds, then place it in the cooler.
19. Immediately add the 50X TITANIUM™ Taq DNA Polymerase Mix to PCR Master Mix tube, then return the 50X TITANIUM™ Taq DNA Polymerase Mix to the cooler.
20. Vortex the PCR Master Mix *continuously* for 3 seconds, then pour it into the reagent reservoir.
21. Remove the seal from the **PCR Plate**.
22. Use a multichannel P200 pipette to add 90 μ L of the PCR Master Mix to each diluted-ligated sample in the **PCR Plate**.

Note: Avoid contamination by changing pipette tips after each transfer.

Sample	Volume/sample
Ligated-Diluted DNA	10.0 μ L
PCR Master Mix	90.0 μ L
Total volume	100.0 μL



23. Seal the **PCR Plate**, then vortex the plate. Repeat the vortex, then centrifuge.
24. While the **PCR Plate** is in the centrifuge, clean up the Pre-PCR Room benchtop.
25. When the centrifuge is complete, place the **PCR Plate** on ice, then transfer the plate to the Post-PCR Room.
26. In the Post-PCR Room, load the **PCR Plate** in the thermal cycler and run the CytoScan Accel PCR protocol.

Temperature	Time	Cycles
94°C	3 minutes	1X
94°C	30 seconds	25X
60°C	45 seconds	
68°C	15 seconds	
68°C	7 minutes	1X
4°C	∞	

27. While the CytoScan Accel PCR protocol is running, prepare the Post-PCR Room:
 - a. Fill the ice bucket with ice.
 - b. Place the cooling block on ice.
 - c. Label a 96-well plate “PCR Gel”, then place it on the lower half of the cooling block. This plate is called the **PCR Gel Plate**.
 - d. Assemble the E-Gel™ Power Snap Plus Electrophoresis System.
 - e. Place an E-Gel™ Agarose Gel with SYBR™ Safe DNA Gel Stain, 2% on the benchtop.
 - f. Place Nuclease-Free Water (from different source), TrackIt™ Cyan/Orange Loading Buffer, and 25 bp DNA Ladder on the benchtop.
28. Prepare dilutions of the TrackIt™ Cyan/Orange Loading Buffer and the 25 bp DNA Ladder.
 - a. Label a 15-mL tube “1:100 diluted Loading Buffer”. Add 100 µL of TrackIt™ Cyan/Orange Loading Buffer to 9.9 mL Nuclease-Free Water (from different source). Mix well, then store at room temperature.
 - b. Label a 1.5-mL tube “1:6 25 bp DNA Ladder”. Add 15 µL of 25 bp DNA Ladder to 75 µL of Nuclease-Free Water (from different source). Vortex, centrifuge, then place it on the benchtop.

29. When CytoScan Accel PCR protocol is complete, take the **PCR Plate** out of the thermal cycler and place it on the cooling block. Centrifuge the plate, then place it on the upper half of the cooling block.
30. Immediately start “Stage 4: In-process QC—PCR gel” on page 5.

Stage 4: In-process QC—PCR gel

Perform the following steps in the Post-PCR Room.

1. Add 17 µL of 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer to wells A1—A8 of the **PCR Gel Plate**.
2. Remove the seal from the **PCR Plate**. Use a multichannel P20 pipette to transfer 3 µL from each sample of the **PCR Plate** to the corresponding well of the **PCR Gel Plate**.
3. Seal the **PCR Plate**, then place it on the upper half of the cooling block.
4. Seal the **PCR Gel Plate**. Vortex, centrifuge, then place it on a plate storage rack.
5. Remove the comb from the gel and insert the gel into the electrophoresis device.
6. Remove the seal from the **PCR Gel Plate**.
7. Load 20 µL of each sample from the **PCR Gel Plate** into the gel wells.
8. Load 15 µL of the 1:6 diluted 25 bp DNA Ladder into the marker wells. Load 15 µL of the 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer into any remaining empty wells.
9. Select the number of wells and the type of E-Gel™ Agarose Gel being run. Set the duration to 21 minutes and start the run.
10. Immediately start “Stage 5: PCR product purification” on page 6.

Note: After 21 minutes, return to the E-Base™ device to inspect and capture an image of the gel. The Sample lanes should have a smear of 150—2,000 bp. The negative control should not have a smear. The image should look like the following example. (Figure 2.)

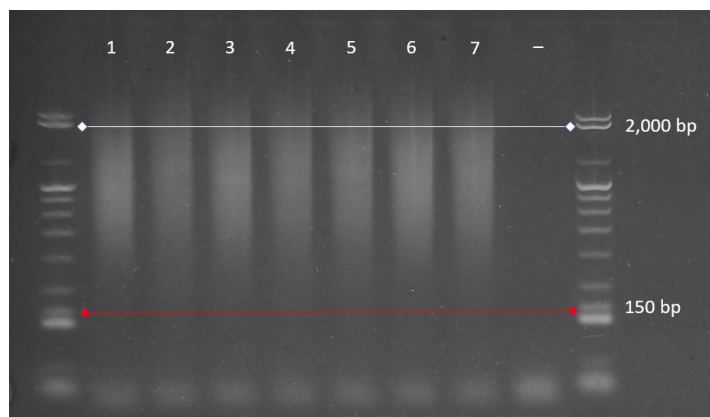


Figure 2 PCR gel image with negative control.

Stage 5: PCR product purification

Perform the following steps in the Post-PCR Room.

1. Prepare the Post-PCR Room:
 - a. Label a 1.6-mL square storage plate "Purification". This plate is called the **Purification Plate**.
 - b. Place the Purification Wash Buffer, Purification Beads, and Elution Buffer on the benchtop.
 - c. Set the microplate shaker to 1,100 rpm.
 - d. Place the Magnum FLX™ Enhanced Universal Magnet Plate on the benchtop.

2. Prepare the Purification Wash Buffer.

- a. Check if the absolute ethanol has been added to the the Purification Wash Buffer. If not, add 45 mL of absolute ethanol. Label the bottle to indicate the addition.
- b. Mix by inverting the bottle 10 times.

IMPORTANT! Ensure the absolute ethanol has been added to the Purification Wash Buffer bottle.

3. Pool the PCR reactions:

- a. Remove the **PCR Plate** from the cooling block, place it on a plate storage rack, then remove the seal.
- b. Use a multichannel P200 pipette set to 100 µL to pool the 4 PCR reaction for each sample. Transfer 4 x 100 µL of each sample from the **PCR Plate** into the corresponding well of the **Purification Plate**. (Figure 3.)
- c. Do not pool the negative control.

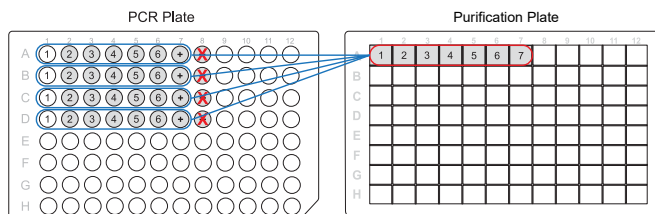


Figure 3 PCR Plate is on left. Purification Plate is on right. Negative control is not pooled.

Note: After the 21 minute timer completes, return to the E-Base™ device to inspect the PCR QC Gel, then capture an image.

4. Thoroughly mix the Purification Beads stock bottle by inverting the bottle 10 times. Ensure the solution is homogenous, then add 5.5 mL into a reagent reservoir.
5. Use a multichannel P1200 pipette to add 600 µL of Purification Beads to each sample in the **Purification Plate**.
6. Use a multichannel P1200 pipette set to 600 µL to mix each row of samples in the **Purification Plate**. Slowly pipet up and down 15 times.
7. Cover the **Purification Plate** with an unopened seal, then incubate for 10 minutes at room temperature on the benchtop.
8. Place the **Purification Plate** on the magnet.

9. Incubate for 10 minutes at room temperature. Beads form a ring at the bottom of each sample well. (Figure 4.)

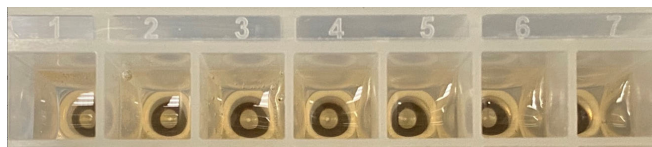


Figure 4 Beads form a ring at the bottom of each sample well.

10. Use a multichannel P1200 pipette set to 850 µL to remove the majority of the supernatant from the **Purification Plate**. Aspirate, then discard the supernatant.

Note: Tips should not touch the bottom of the well to prevent disturbing the beads.

Note: There will be ~150 µL of supernatant remaining in each well.

11. Use a multichannel P200 pipette set to 200 µL to remove the remaining supernatant from the **Purification Plate**. Aspirate, then discard the supernatant.

Note: Position the pipette tips toward the center of the bead ring. The tips can touch the bottom of the sample well.

12. Move the **Purification Plate** off the magnet and onto the benchtop.

13. Add 11 mL of Purification Wash Buffer into a reagent reservoir.

14. Use a multichannel P1200 pipette to add 400 µL of Purification Wash Buffer to each sample in the **Purification Plate**.

Note: Do not discard the remaining Purification Wash Buffer in the reagent reservoir. Cover it with an unopened seal.

15. Seal the **Purification Plate**, then place it on the microplate shaker. Shake the plate at 1,100 rpm for 2 minutes.

16. Move the **Purification Plate** from the microplate shaker onto the benchtop. Remove the seal from the **Purification Plate**.

17. Place the **Purification Plate** on the magnet.

18. Incubate for 3 minutes at room temperature. Beads form an irregular ring at the bottom of each sample well. (Figure 5.)

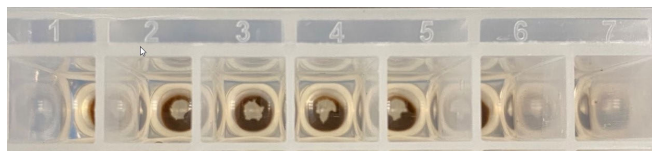


Figure 5 Beads form an irregular ring at the bottom of each sample well.

19. Use a multichannel P200 pipette set to 200 µL to remove the supernatant from the **Purification Plate**. Aspirate, then discard the supernatant.

Note: Tips should not touch the bottom of the sample well to prevent disturbing the beads.

Note: There will be ~200 µL of supernatant remaining in each well.

20. Move the **Purification Plate** off the magnet and onto the benchtop.
21. Use a multichannel P1200 pipette to add 1,000 μL of Purification Wash Buffer to each sample well of the **Purification Plate**.
22. Place the **Purification Plate** on the magnet. Incubate the plate for 1 minute at room temperature. Beads form an irregular ring at the bottom of each sample well.
23. Use a multichannel P1200 pipette set to 1,000 μL to remove the supernatant from the **Purification Plate**. Aspirate, then discard the supernatant.

Note: Tips should not touch the bottom of the sample well to prevent disturbing the beads.

Note: There will be ~ 200 μL of supernatant remaining in each well.
24. Use a multichannel P200 pipette set to 200 μL to remove the remaining supernatant from the **Purification Plate**. Aspirate, then discard the supernatant.

Note: Tips can touch the bottom of the sample well. If the bead ring covers the center area, use the pipette tip to push the beads to the side of the sample well. Aspirate, then discard the supernatant.
25. Use a multichannel P20 pipette set to 20 μL to remove any remaining supernatant from the **Purification Plate**. Aspirate, then discard the supernatant.

Note: Tips can touch the bottom of the sample well.

IMPORTANT! Ensure that there is no liquid remaining in any sample well of the **Purification Plate**.

26. Move the **Purification Plate** off the magnet and onto the benchtop. Air dry the **Purification Plate** for 5 minutes at room temperature. Do not cover the plate with a seal.

IMPORTANT! Do not allow the beads to dry longer than 5 minutes.
27. Vortex the Elution Buffer. Place a strip tube on a plate storage rack. Add 70 μL of Elution Buffer into wells 1–7.
28. Use a multichannel P200 pipette to add 52 μL of Elution Buffer to each sample well of the **Purification Plate**.

Note: Position the pipette tips in the center of the sample well, just above the beads. Dispense directly onto the beads.
29. Seal the **Purification Plate**, then place it on the microplate shaker. Shake the plate at 1,100 rpm for 10 minutes.
30. While the **Purification Plate** is shaking, prepare the Post-PCR Room:
 - a. Power on the NanoDrop™ Spectrophotometer.
 - b. Set the plate centrifuge to 4°C to cool to temperature.
 - c. Power on the thermal cycler in the Post-PCR Room to preheat the lid.
 - d. Refresh ice in the ice bucket.
 - e. Place the cooling block on ice.

31. Prepare the PCR quantitation:
 - a. Place the Nuclease-Free Water on the benchtop.
 - b. Label a 96-well plate, “OD”, then place it on a plate storage rack. This plate is called the **OD Plate**.
 - c. Prepare the Sample Dilution row by adding 18 μL of Nuclease-Free Water to wells A1–A7 of the **OD Plate**.
 - d. Prepare the NanoDrop™ Blank by adding 18 μL of Nuclease-Free Water to well C1 of the **OD Plate**.
 - e. Seal the **OD Plate**.
32. Prepare the Fragmentation reagents:
 - a. Place the Nuclease-Free Water on ice.
 - b. Thaw the 10X Fragmentation Buffer at room temperature.
 - c. Once thawed, place it on ice.

Note: Fragmentation reagents are vortexed and centrifuged in later steps.
33. When the 10 minute shaking step is complete, move the **Purification Plate** from the microplate shaker onto the benchtop.
34. Remove the seal from the **Purification Plate**.
35. Place the **Purification Plate** on the magnet. Incubate for 1 minute at room temperature. The beads form a ring at the bottom of each sample well. (Figure 6.)

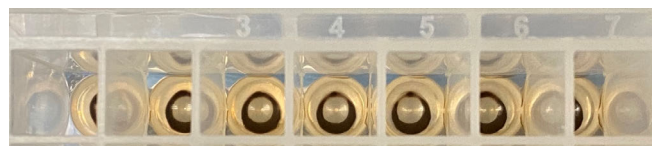


Figure 6 Beads form a ring at the bottom of each sample well.

36. Label a 96-well plate “Fragmentation”, then place it on a plate storage rack.

This plate is called the **Fragmentation Plate**.
37. Use a multichannel P200 pipette set to 47 μL to remove the purified PCR product from the **Purification Plate**. Aspirate, then dispense to the corresponding well of the **Fragmentation Plate**.

IMPORTANT! Do not discard the purified PCR product.

Note: Tips can touch the bottom of the sample well.
38. Seal the **Fragmentation Plate**. Vortex, centrifuge, then place it on the lower half of the cooling block.
39. Immediately start “Stage 6: In-process QC—Quantitation of PCR product” on page 8.

Stage 6: In-process QC—Quantitation of PCR product

Perform the following steps in the Post-PCR Room.

1. Remove the seal from the **Fragmentation Plate** and the **OD Plate**.
2. Complete the Sample Dilution row. Use a multichannel P20 pipette to transfer 2 μL from the **Fragmentation Plate** to the corresponding wells of the **OD Plate**. Pipet up and down 2 times to rinse the tips.

3. Seal the **Fragmentation Plate**, then place it on the lower half of the cooling block.

IMPORTANT! It is critical that the **Fragmentation Plate** remain on the cooling block.

4. Seal the **OD Plate**, vortex, then centrifuge.
5. Quantitate the **OD Plate** using the NanoDrop™ Spectrophotometer. Blank the NanoDrop™, then measure the OD of each sample at 260, 280, and 320 nm.
6. Determine the acceptable DNA yield for each sample and all samples.
 - For each sample, the DNA yield must be $\geq 1.8 \mu\text{g}/\mu\text{L}$.
7. Determine the acceptable DNA purity for each sample.
 - $\text{OD}_{260}/\text{OD}_{280}$ ratio = 1.7 to 2.1
 - OD_{320} measurement is close to zero (≤ 0.1)
8. If the Purified PCR product meets the requirements, immediately start “Stage 7: Fragmentation” on page 8.

Stage 7: Fragmentation

Perform the following steps in the Post-PCR Room.

1. Prepare the Post-PCR Room:
 - a. Ensure that the thermal cycler is powered on and the lid is preheated.
 - b. Ensure that the centrifuge is chilled.
 - c. Ensure that the **Fragmentation Plate**, reagents, and consumables are chilled before proceeding.
2. Place an 8-well strip tube on the cooling block. Label a 1.5-mL tube “Frag”, then place it on the cooling block.
3. Prepare the 10X Fragmentation Buffer. Vortex, centrifuge, then place it on the cooling block.

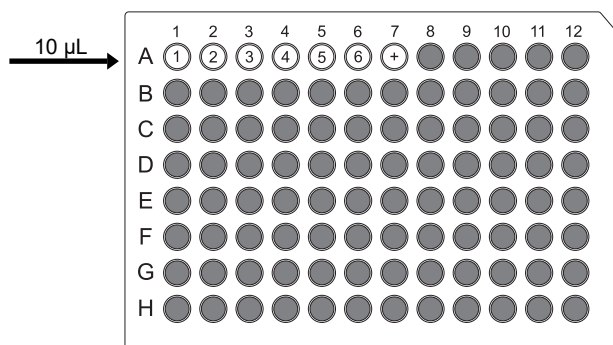
4. Prepare the Fragmentation Master Mix as shown in the following table. Add chilled Nuclease-Free Water and 10X Fragmentation Buffer to the 1.5-mL tube labeled “Frag”. Vortex, centrifuge, then place it on the cooling block.

Table 4 Fragmentation Master Mix.

Reagent	Volume
Nuclease-Free Water	271.2 μL
10X Fragmentation Buffer	343.8 μL
Fragmentation Reagent	10.0 μL
Total	625.0 μL

5. Remove the Fragmentation Reagent from the freezer and immediately place it in a cooler. Centrifuge for 1 second, then vortex 1 second. Centrifuge for 3 seconds, then place it in the cooler.
6. Immediately add the Fragmentation Reagent to the Fragmentation Master Mix tube, then return the Fragmentation Reagent to the cooler.
7. Vortex and centrifuge the Fragmentation Master Mix, then place it on the cooling block.
8. Aliquot the Fragmentation Master Mix into the strip tube. Add 40 μL into wells 1–7. Place the strip tube on the cooling block.
9. Remove the seal from the **Fragmentation Plate**.
10. Use a multichannel P20 pipette to add 10 μL of the Fragmentation Master mix to each sample in the plate.

Samples	Volume/sample
Purified PCR product	45.0 μL
Fragmentation Master Mix	10.0 μL
Total volume	55.0 μL



This plate is still called the **Fragmentation Plate**.

11. Seal the **Fragmentation Plate**, vortex, then place it on the cooling block.
12. Transfer the **Fragmentation Plate** to the pre-chilled 4°C centrifuge. Centrifuge the plate, then place it on the cooling block.

13. Ensure that the thermal cycler lid is preheated, if not, keep the **Fragmentation Plate** on the cooling block, then preheat the thermal cycler lid.

14. Load the **Fragmentation Plate** in the thermal cycler and run the CytoScan Accel Fragmentation protocol.

Temperature	Time
37°C	35 minutes
95°C	15 minutes
4°C	∞

15. While the CytoScan Accel Fragmentation protocol is running, prepare the Post-PCR Room:

- a. Set the centrifuge to room temperature and allow it to equilibrate.
- b. Label a 96-well plate “Frag Gel”, then place it on the lower half of the cooling block. This plate is called the **Frag Gel Plate**.
- c. Assemble the E-Gel™ Power Snap Plus Electrophoresis System.
- d. Place an E-Gel™ Agarose Gel with SYBR™ Safe DNA Gel Stain, 4% on the benchtop.
- e. Place the previously prepared 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer and the 1:6 diluted 25 bp DNA Ladder on the benchtop.

16. When the CytoScan Accel Fragmentation protocol is complete, take the **Fragmentation Plate** out of the thermal cycler and place it on the cooling block. Centrifuge the plate, then place it on the upper half of the cooling block.

17. Immediately start “Stage 8: In-process QC—Fragmentation gel” on page 9.

Stage 8: In-process QC—Fragmentation gel

Perform the following steps in the Post-PCR Room.

1. Add 76 µL of 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer to wells A1 —A7 of the **Frag Gel Plate**.
2. Remove the seal from the **Fragmentation Plate**. Use a multichannel P20 pipette to transfer 4 µL from each sample of the **Fragmentation Plate** to the corresponding well of the **Frag Gel Plate**.
3. Seal the **Fragmentation Plate**, then place it on the lower half of the cooling block.
4. Seal the **Frag Gel Plate**. Vortex, centrifuge, then place it on a plate storage rack.
5. Remove the comb from the gel and insert the gel into the electrophoresis device.
6. Remove the seal from the **Frag Gel Plate**.
7. Load 20 µL of each sample from the **Frag Gel Plate** into the gel wells.
8. Load 15 µL of the 1:6 diluted 25 bp DNA Ladder into the marker wells. Load 15 µL of the 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer into any remaining empty wells.
9. Select the number of wells and the type of E-Gel™ Agarose Gel being run. Set the duration to 19 minutes and start the run.
10. Immediately start “Stage 9: Labeling” on page 10.

Note: After 19 minutes, return to the E-Base™ device to inspect then capture an image of the gel. The sample lanes should have a smear of 25 - 125 bp. The image should look like the following example. (Figure 7.)

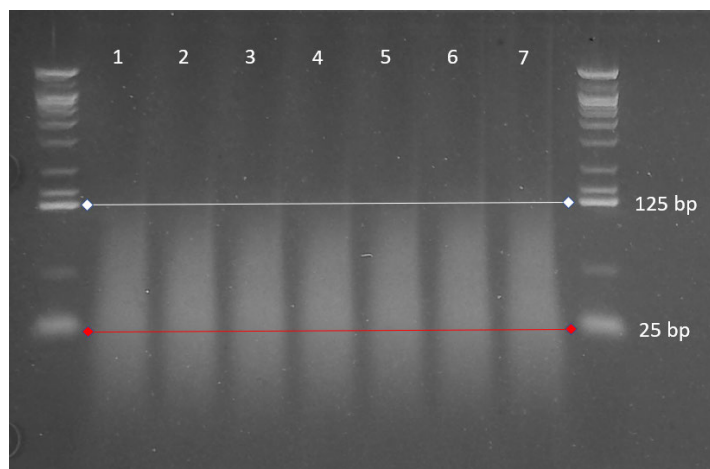


Figure 7 Fragmentation gel image.

Stage 9: Labeling

Perform the following steps in the Post-PCR Room.

1. Prepare the Labeling reagents:
 - a. Thaw the TdT Buffer and DNA Labeling Reagent at room temperature.
 - b. Once thawed, place them on ice.

Note: Labeling reagents are vortexed and centrifuged in later steps.

2. Prepare the Hybridization reagents:
 - a. Thaw the Hyb Buffer Part 1, Hyb Buffer Part 2, Hyb Buffer Part 3, Hyb Buffer Part 4, and Oligo Control Reagent at room temperature.
 - b. Once thawed, place them on ice.




Note: Hybridization reagents are vortexed and centrifuged in later steps.

3. Place an 8-well strip tube on the cooling block. Label a 1.5-mL tube “LBL”, then place it on the cooling block.

Note: After the 19-minute timer completes, return to the E-Base™ device to inspect the Fragmentation Gel, then capture an image. Do not prepare the Labeling Master Mix until the Fragmentation Gel confirms the correct fragment size.

4. Prepare the TdT Buffer and DNA Labeling Reagent. Vortex, centrifuge, then place them on the cooling block.
5. Prepare the Labeling Master Mix as shown in the following table. Add TdT Buffer and DNA Labeling Reagent to the 1.5-mL tube labeled “LBL”. Vortex, centrifuge, then place it on the cooling block.

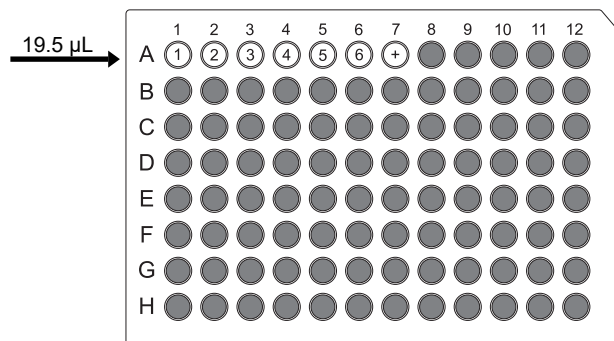
Table 5 Labeling Master Mix (≥8 samples, 20% overage).

Reagent	1 sample	8 samples
 TdT Buffer	14.0 µL	134.4 µL
 DNA Labeling Reagent	2.0 µL	19.2 µL
 TdT Enzyme	3.5 µL	33.6 µL
Total	19.5 µL	187.2 µL

6. Remove the TdT Enzyme from the freezer and immediately place it in a cooler. Centrifuge for 1 second, then vortex 1 second. Centrifuge for 3 seconds, then place it in the cooler.
7. Immediately add the TdT Enzyme to the Labeling Master Mix tube, then return the TdT Enzyme to the cooler.
8. Vortex and centrifuge the Labeling Master Mix, then place it on the cooling block.
9. Aliquot the Labeling Master Mix into the strip tube. Add 22 µL into wells 1–7. Place the strip tube on the cooling block.
10. Remove the seal from the **Fragmentation Plate**.

11. Use a multichannel P20 pipette to add 19.5 µL of the Labeling Master Mix to each sample in the plate.

Sample	Volume/sample
Fragmented DNA	51.0 µL
Labeling Master Mix	19.5 µL
Total volume	70.5 µL



The plate is now called the **Labeling Plate**.

12. Seal the **Labeling Plate**, vortex, then centrifuge.
13. Load the **Labeling Plate** in the thermal cycler and run the CytoScan Accel Label protocol.

Temperature	Time
37°C	30 minutes
95°C	15 minutes
4°C	∞

14. While the CytoScan Accel Label protocol is running, prepare the arrays and equipment:
 - a. Remove arrays from 4°C and allow them to warm to room temperature.
 - b. Power on the GeneChip™ Hybridization Oven 645 and set it to 50°C and 60 rpm.
 - c. Allow the oven to operate for 30 minutes before arrays are loaded.
15. Register the arrays using GCDC.
16. When CytoScan Accel Label protocol is complete, take the **Labeling Plate** out of the thermal cycler and place it on the cooling block. Centrifuge the plate, then place it on the lower half of the cooling chamber.
17. Immediately start “Stages 10 and 11: Hybridization Cocktail and Array Hybridization” on page 11.

Stages 10 and 11: Hybridization Cocktail and Array Hybridization

Perform the following steps in the Post-PCR Room.

1. Prepare the Post-PCR Room:
 - a. Ensure that the thermal cycler is powered on.
 - b. Refresh ice in the ice bucket.
 - c. Place the cooling block on ice.
 - d. Place a reservoir on the top half of the cooling block.
2. Label a 15-mL tube "Hyb", then place it on ice.
3. Prepare the Hyb Buffer Part 1. Vortex, then place it on ice.
4. Prepare the Hyb Buffer Part 2, Hyb Buffer Part 3, Hyb Buffer Part 4, and the Oligo Control Reagent. Vortex, centrifuge, then place them on the cooling block.
5. Prepare the Hybridization Master Mix as shown in the following table. Add Hyb Buffer Part 1, Hyb Buffer Part 2, Hyb Buffer Part 3, Hyb Buffer Part 4, and the Oligo Control Reagent to the 15-mL tube labeled "Hyb".

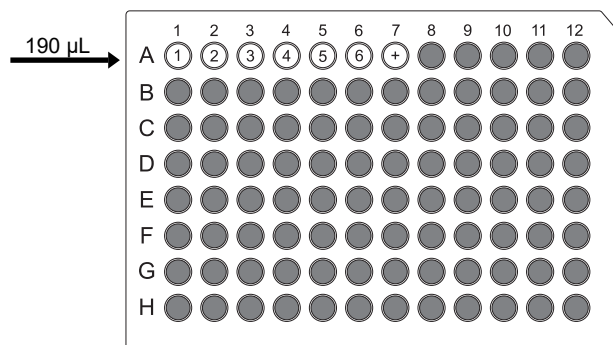
Table 6 Hybridization Master Mix (≥8 samples, 20% overage).

Reagent and cap color	1 sample	8 samples
● Hyb Buffer Part 1	165.0 µL	1,584.0 µL
● Hyb Buffer Part 2	15.0 µL	144.0 µL
● Hyb Buffer Part 3	7.0 µL	67.2 µL
● Hyb Buffer Part 4	1.0 µL	9.6 µL
● Oligo Control Reagent	2.0 µL	19.2 µL
Total volume	190.0 µL	1,824.0 µL

6. Vortex the Hybridization Master Mix *continuously* for 3 seconds. Repeat the vortexing step 2 more times.
7. Pour the Hybridization Master Mix into the reagent reservoir. Remove the seal from the **Labeling Plate**.
8. Use a multichannel P200 pipette to add 190 µL of Hybridization Master Mix to each sample in the plate.

Table 7 Hybridization Plate well volumes.

Sample	Volume/sample
Labeled DNA	70.5 µL
Hybridization Master Mix	190.0 µL
Total volume	260.5 µL



The plate is now called the **Hybridization Plate**.

9. Seal the **Hybridization Plate**, then vortex the plate. Repeat the vortex, then centrifuge.

IMPORTANT! Ensure that the plate is vortexed well to mix the sample and hybridization buffer.

10. Load the **Hybridization Plate** in the thermal cycler and run the CytoScan Accel Hyb protocol.

Temperature	Time
95°C	10 minutes
49°C	3 minutes
49°C	∞

11. While the CytoScan Accel Hyb protocol is running, prepare the arrays:
 - a. Place the arrays on a clean benchtop close to the thermal cycler and the hybridization oven.
 - b. Insert a 200-µL pipette tip into the upper right septum of each array.
 - c. Paste two 1/2" Tough-Spots™ label dots on the top edge of each array.
 12. When the CytoScan Accel Hyb Protocol reaches the 49°C ∞ step, open the lid. Keep the **Hybridization Plate** in the thermal cycler.
 13. Use a blade to cut the seal of the **Hybridization Plate** between rows.
 14. Use a P200 pipette to remove 200 µL of a Hybridization Cocktail from the **Hybridization Plate**, then immediately inject it into the corresponding array.
 15. Cover the septa of each array with 1/2" Tough-Spots™ label dots. Immediately load the arrays into the GeneChip™ Hybridization Oven 645.
- IMPORTANT!** Only process 4 arrays at a time. Do not allow injected arrays to sit at room temperature for more than 1 minute.
16. Repeat step 13—step 15 until all arrays are injected and loaded into the hybridization oven.

17. Allow the arrays to hybridize for 16—18 hours at 50°C and 60 rpm.

IMPORTANT! The hybridization time, temperature, and rotational speed are optimized for this product and must be stringently followed.

18. When all arrays are loaded into the oven, remove the **Hybridization Plate** from the thermal cycler, seal, then store the plate at –25°C to –15°C.
19. After 15.5 hours, proceed to “Stages 12 and 13: Wash, stain, and scan the arrays” on page 12.

Stages 12 and 13: Wash, stain, and scan the arrays

Perform the following steps in the Post-PCR Room.

1. Prepare the stains:
 - a. Mix Stain Buffer 1, Stain Buffer 2, and Array Holding Buffer by gently inverting them 10 times.
 - b. For each array, aliquot:
 - 500 µL of Stain Buffer 1 into an amber tube.
 - 500 µL of Stain Buffer 2 into a clear tube.
 - 800 µL of Array Holding Buffer into a blue tube.
2. Prepare the GeneChip™ Fluidics Station 450:
 - a. Place full bottles of Wash A, Wash B, and DI water on the fluidics station.
 - b. Empty the Waste Bottle and place it on the fluidics station.
3. Prime the fluidics station. When the protocol is complete, eject the washblock to avoid a sensor timeout.
4. Load the Stain Buffers and the Array Holding Buffer onto the fluidics station.
5. After 16—18 hours, remove arrays from the hybridization oven. Remove the Tough-Spots™ label dots, then load the arrays into the fluidics station.

IMPORTANT! Arrays should not be in the hybridization oven longer than 18 hours.

6. Run the **CytoScanHD Accel_450** fluidics protocol.
7. When the protocol is complete, remove the arrays from the fluidics station. Check for bubbles.
8. Cover the septa of each array with 3/8" Tough-Spots™ label dots. Begin scanning the arrays.
9. When the hybridization of all arrays is complete, power off the oven.
10. When the washing and staining of all arrays is complete, perform the **Shutdown** protocol, then power off the fluidics station.
11. When all arrays have been scanned, close the GCDC software, then power off the scanner.



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Products manufactured at this site:
CytoScan™ HD Accel Array



Thermo Fisher Scientific Baltics UAB |
V.A. Graiciuno 8, LT-02241 |
Vilnius, Lithuania

Products manufactured at this site:
CytoScan™ Accel Reagent Kit

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0028087

Revision	Date	Description
A.0	11 July 2023	Initial release to support the CytoScan™ Accel Assay Manual Workflow.

The information in this guide is subject to change without notice.

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