February 2023 Quick-Start Protocol

EpiTect Hi-C Kit

The contents of the EpiTect Hi-C Kit (cat. no. 59971) should be stored immediately upon receipt at the following temperatures: Box 1 at –15 to –30°C in a constant-temperature freezer, Box 2 at room temperature (15–25°C), and Box 3 at 2–8°C.

Further information

- *EpiTect Hi-C Handbook*: **www.qiagen.com/HB-2625**
- *EpiTect Hi-C Data Analysis Portal User Guide*: **www.qiagen.com/HB-2631**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Be sure to have all user supplied reagents on hand before starting the protocol
- The EpiTect Hi-C procedure is optimized use with 5 x 10^5 human or mouse cells (or the equivalent of 3 µg of DNA) per sample. However, it can be used with down to 5 x 10^3 human or mouse cells (or the equivalent of 30 ng of DNA). Refer to the *EpiTect Hi-C Kit Handbook* for more information.

Hi-C Part 1: Processing Fixed Cells Into a Hi-C Library

Table 1. Preparing solutions for Hi-C Part 1

Hi-C Digestion Solution		Hi-C Ligation Solution	
Component	**Volume**	**Component**	**Volume**
Hi-C Digestion Buffer	4 µL	Hi-C Ligation Buffer	200 µL
1% SDS	4 µL	10% Triton X-100	40 µL
RNase-free water	32 µL	Ultralow Input Ligase	5 µL
–	–	RNase-free water	105 µL
Total volume	**40 µL**	**Total volume**	**350 µL**

1. Resuspend crosslinked cells in 50 µL of ice cold PBS.
2. Add 150 µL cold RNase-free water and 50 µL cold Buffer C1.
3. Gently mix by inverting, and then incubate on ice for 10 min.
4. Add 250 µL of QIAseq Beads to the sample. Mix by gently inverting and let stand at room temperature for 10 min.
5. Wash QIAseq Beads once with 500 µL cold RNase-free water.
6. Resuspend QIAseq Beads in 40 µL Hi-C Digestion solution.
7. Incubate tube at 65°C for 10 min, and then place on ice.
8. Add 4.4 µL 10% Triton X-100 and 4 µl Hi-C Digestion Enzyme. Mix by pipetting.
9. Incubate tube at 37°C with gentle shaking (600 rpm) for 30 min.
10. Incubate tube at 65°C for 20 min, and then place on ice.
11. Add 6 µL of Hi-C End Labeling Mix, and mix by gentle pipetting.
12. Add 1 µL of Hi-C End Labeling Enzyme, and mix by gentle pipetting.
13. Incubate at 37°C for 30 min, and then place on ice.
14. Add 350 µL of Hi-C Ligation Solution, and gently mix by inverting.
15. Incubate tube at 16°C for 30 min, and then place on ice.
16. Add 10 µL of Proteinase K to tube, and gently mix by inverting.
17. Incubate tube at 56°C for 30 min, followed by 80°C for 90 min.
18. Cool tube to room temperature (RT).
19. Add 40 µL of 3 M sodium acetate, pH 5.2, to tube. Vortex briefly.
20. Add 280 µL of 100% isopropanol to tube. Vortex briefly.
21. Apply entire mixture, including QIAseq Beads, to a MinElute® column. Centrifuge for 1 min at 17,900 × *g*. Discard flow-through.
22. Add 0.75 mL Buffer PE to column, centrifuge for 1 min at 17,900 × *g*, discard flow-through, and return column to the same tube.
23. Centrifuge the column for an additional 1 min at 17,900 × *g*.
24. Place column into a new 1.5 mL microcentrifuge tube.
25. Add 35 µL of Buffer EB warmed to 65°C to the membrane. Incubate for 1 min.
26. Centrifuge column for 1 min at 17,900 × *g* to elute DNA.
27. Store purified DNA at –20°C or proceed with Hi-C Part 2.

Hi-C Part 2: Processing a Hi-C Library Into an NGS Sequencing Library

Table 2. Preparing solutions for Hi-C Part 2

ER/A-tailing solution		Adapter ligation buffer dilution		Hi-C sequencing library amplification mix	
Component	**Volume**	**Component**	**Volume**	**Component**	**Volume**
ER/A-Tailing Buffer	5 µL	Adapter Ligation Buffer	15 µL	HiFi PCR Master Mix, 2x	75 µL
ER/A-Tailing Enzyme Mix	10 µL	RNase-free water	135 µL	Primer Mix Illumina Library Amp	4.5 µL
RNase-free water	35 µL	–	–	RNase-free water	70.5 µL
Total volume	**50 µL**	**Total volume**	**150 µL**	**Total volume**	**150 µL**

28. Fragment DNA from Hi-C Part 1 to a median size of 400–600 bp.
29. Add 4 volumes Buffer SB1 to 1 volume of DNA. Vortex briefly.
30. Apply mixture to a MinElute column. Centrifuge for 1 min at 17,900 x *g*.
31. Discard flow-through and return column to the same collection tube.
32. Add 700 µL 80% ethanol to column. Centrifuge for 1 min at 17,900 x *g*.
33. Discard flow-through and return column to the same collection tube.
34. Repeat steps 5–6. Centrifuge column again for 1 min at 17,900 x *g*.
35. Place column in new microcentrifuge tube. Add 50 µL Buffer EB warmed to 65°C to the membrane and incubate for 1 min (RT).
36. Centrifuge column for 1 min at 17,900 x *g* to elute DNA.
37. Transfer 25 µL streptavidin beads into a new microcentrifuge tube.
38. Wash beads once in 100 µL Bead Wash Buffer.
39. Resuspend beads in 50 µL Bead Resuspension Buffer. Add the 50 µL of purified DNA and incubate for 15 min (RT) with shaking (1000 rpm).
40. Wash beads once in 100 µL Bead Wash Buffer 2.
41. Resuspend beads in 50 µL of prepared ER/A-tailing solution. Incubate for 15 min at 20°C, followed by 15 min at 65°C.
42. Wash beads once in 100 µL Bead Wash Buffer 2.
43. Wash beads once in 95 µL diluted adapter ligation buffer.
44. Resuspend beads in 50 µL diluted adapter ligation buffer.
45. Transfer 5 µL of one Illumina® Adapter well to a sample tube.

46. Add 2 µL ultralow input ligase, mix by pipetting, and incubate for 45 min (RT).
47. Wash the beads twice with 100 µL Bead Wash Buffer 1, then twice with 100 µL Bead Wash Buffer 2, and finally with 100 µL RNase-free water.
48. Add 150 µL of Hi-C sequencing library amplification mix. Vortex briefly to mix.
49. Amplify the NGS library using the cycling program described in the handbook.
50. Pull down streptavidin beads in magnetic rack. Transfer supernatant to fresh microcentrifugation tube. Store at –20°C or proceed to next step.
51. Add 150 µL of QIAseq® Beads, equilibrated to RT, to the supernatant. Vortex briefly and incubate for 5 min (RT).
52. Wash QIAseq beads twice in 500 µL 80% ethanol.
53. Briefly microcentrifuge the tube at 5000 x *g* (RT). Transfer to magnetic rack, incubate for 30 s, and then remove supernatant.
54. Incubate beads with lid open for 2–5 min (RT) until dry.
55. Remove tube from rack. Resuspend beads in 25 µL EB buffer. Incubate for 1 min (RT). Place tube back in the magnetic rack and for incubate 1 min (RT).
56. Transfer supernatant containing NGS library to a fresh microcentrifuge tube.
57. Proceed directly to "Hi-C sequencing library quality control and quantification" as specified in the EpiTect Hi-C Kit Handbook, or store NGS library at –20°C.

Document Revision History

Date	Changes
04/2019	Initial release
02/2023	Updated to new brand template. Additional information in notes and steps.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.