ZYMO RESEARCH

Epigenetics *Made Simple*™

EZ DNA Methylation-Lightning™ Automation Kit

Automated Bisulfite Conversion for Single Base Pair Resolution Methylation Analysis

Highlights

- Automation-specific streamlined design for high-throughput bisulfite conversion of DNA for methylation analysis.
- Compatible with Illumina Infinium™ MethylationEPIC, MethylationEPIC+, HTS iSelect® Methyl Custom, Mouse Methylation and Mouse Methylation+ BeadChips.
- Optimized buffer volumes for consistent automation performance.

Catalog Numbers:
D5049

Scan with your smart-phone camera to view the online protocol/video.

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Product Contents

EZ DNA Methylation-Lightning™ Automation Kit	D5049 (96 preps)	Storage Temperature
Lightning Conversion Reagent[1]	15 ml	Room Temp.
M-Binding Buffer	100 ml	Room Temp.
M-Wash Buffer[2]	36 ml	Room Temp.
L-Desulphonation Buffer	80 ml	Room Temp.
M-Elution Buffer	50 ml	Room Temp.
EZ-Methylation Magprep Beads	4 ml	Room Temp.
Instruction Manual	1	-

[1]The **Lightning Conversion Reagent** is in a ready-to-use liquid format. The reagent should be stored tightly capped at room temperature with minimum exposure to light.
[2]Add 144 mL of 100% ethanol (156 ml of 95% ethanol) to the 36 ml **M-Wash Buffer** concentrate prior to use.

Product Description

The **EZ DNA Methylation-Lightning™ Automation Kit** offers a high-throughput bisulfite conversion workflow that utilizes magnetic bead-based purification and optimized buffer volumes for consistent automation performance. It is the only recommended automated bisulfite conversion kit for downstream Illumina Infinium™ Methylation BeadChips **(Table 1. Compatible Infinium™ Methylation BeadChip Kits)**. Its streamlined workflow is compatible with all open platform liquid handlers and magnetic bead transfer automated systems. Automation scripts for verified instruments are readily available upon request **(Table 2. Verified Automation Instruments)**.

Table 1. Compatible Infinium™ Methylation BeadChip Kits

Product Description	Illumina Catalog No.
MethylationEPIC BeadChip Kit	20042130 15070021 15070022 15070023
MethylationEPIC+ BeadChip Kit	20020533 20020530 20020531 20020532
Mouse Methylation BeadChip Kit	20041558 20041559 20041560
Mouse Methylation+ BeadChip Kit	20041561 20041562 20041563
HTS iSelect® Methyl Custom BeadChip Kit	20042146

Table 2. Verified Automation Instruments

Instrument	Max No. of Samples	Instrument Run-time
Hamilton® Microlab® STAR™/STARlet™	96	2.5 hours[1]
Tecan® Fluent®	96	2.5 hours[1]
Thermo Scientific™ KingFisher™ Flex	96	1.2 hours[2]

[1] Liquid handler run-times based on workflow using 8-channel pipette and plate-shaker.
[2] KingFisher™ Flex run-time is based on the Automated-Elution script duration.

Reproducible Infinium™ Methylation Array Results

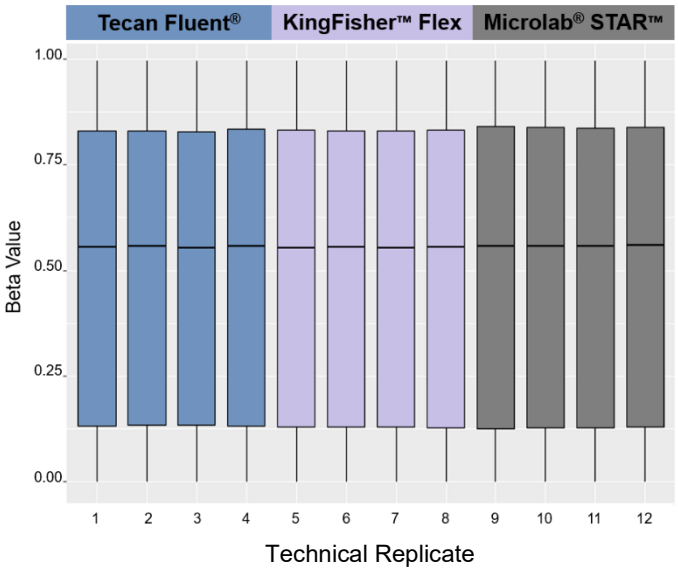


Figure 1. Twelve HeLa cell-line technical replicates were each bisulfite converted on a Tecan® Fluent®, KingFisher™ Flex, or Hamilton® Microlab® STAR™ using the **EZ DNA Methylation-Lightning™ Automation Kit**. The bisulfite converted DNA was then prepared using the Illumina Infinium™ MethylationEPIC BeadChip Kit.

High-Throughput Library Preparation

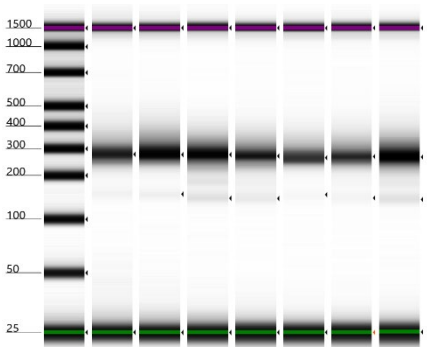


Figure 2. Seven pools each containing 80 samples of targeted bisulfite sequencing libraries were analyzed on the Agilent 2200 Tapestation® HSD1000. Each sample was bisulfite converted on a KingFisher™ Flex using the **EZ DNA Methylation-Lightning™ Automation Kit** followed by library preparation using a Tecan® Fluent®.

Specifications

- **Sample Sources:** Sample containing between 100 pg to 2 µg of purified DNA. For optimal results, the amount of input DNA should be from 200 to 500 ng.
- **Conversion Efficiency:** > 99.5% of non-methylated C residues are converted to U; > 99.5% protection of methylated cytosines.
- **Size and Recovery:** Total DNA (≥ 25 bp)

Size Range	Input Recovery
≥ 100 bp	> 80%
25-100 bp	> 50%

- **DNA Purity:** A260/A280 & A260/A230 ≥ 1.8
- **Binding Capacity:** 1 µg total DNA per 5 µl **EZ-Methylation Magprep Beads**
- **User Provided Materials:** All users will require ethanol (95 – 100%), non-skirted 96-well microplates, and 96-well microplate sealing films. See additional instrument specific hardware/labware recommendations on the following pages:

Hardware/Labware Recommendations	
Instrument	**Page**
Hamilton® Microlab® STAR™/STARlet™	8
Tecan® Fluent® 480	11
Tecan® Fluent® 720/1080	13
Thermo Scientific™ KingFisher™ Flex	16

Protocol Overview

The following is a description of the EZ DNA Methylation-Lightning™ Automation kit's recommended workflow. Sample preparation steps can be automated on liquid handlers upon request.

Manual

1. **Sample Preparation**– 20 µl of purified DNA is mixed with 130 µl of Lightning Conversion Reagent. The samples then undergo the conversion reaction on a thermal cycler. These steps are recommended to perform manually off-deck.
2. **Instrument Set-Up** – Reagents are loaded into troughs and labware is placed on the liquid handler instrument. KingFisher™ Flex users will manually load all reagents into deep well plates.
3. **Sample Transfer** – The sample plate from step 1 is loaded onto the liquid handler instrument where it will be transferred to a binding plate containing 600 µl M-Binding Buffer and 10 µl EZ-Methylation Magprep Beads during the script. KingFisher™ Flex users will manually transfer the samples into the binding plate prior to loading the instrument. The automation scripts start here.

Automated

4. **EZ-Methylation Magprep Bead Binding and Buffer Removal** – The deep well plate containing the sample undergoes mixing for 5 minutes while DNA binds to the beads. The beads are then aggregated using a magnet for 4 minutes and the binding buffer is removed/separated.
5. **M-Wash 1** – 400 µl of M-Wash Buffer is added and the deep well plate is mixed for 1-2 minutes. The beads are then aggregated using a magnet for 2 minutes and the wash buffer is removed/separated.
6. **L-Desulphonation Incubation** – 200 µl of L-Desulphonation Buffer is added and allowed to incubate for 15 minutes. The beads are then aggregated using a magnet for 2 minutes and the desulphonation buffer is removed/separated.
7. **M-Wash 2 & 3** – Step 5 is repeated twice to thoroughly wash the beads. Residual wash buffer is carefully removed to improve drying. KingFisher™ Flex users can stop here and manually perform the remaining steps to ensure minimal yield loss.
8. **Bead Drying** – Beads are left to dry at 55 °C for 20-30 minutes or at room temperature for 30 minutes.
9. **Elution** – 25 µl of M-Elution Buffer is added to the beads and mixed for 5 minutes. The beads are then aggregated using a magnet for 2 minutes and the eluate is transferred to a new 96-well microplate. The scripts end here.

Getting Started

A. Automation Scripts

EZ DNA Methylation-Lightning™ Automation scripts are readily available for verified instruments upon request. For automation scripts and related technical support, email automation@zymoresearch.com. In the subject line, please include the instrument used and the product catalog number (D5049).

The Hamilton® script can accommodate both 8-channel and 96-channel workflows. Tecan® users will have the choice between using an 8-channel workflow or a 96-channel workflow depending on their instrument configuration. KingFisher™ Flex users have a choice between two automated scripts: Manual-Elution or Automated-Elution. For more information about the two different KingFisher™ Flex scripts see page 16.

B. Importing The Script

- ✓ **Hamilton® Microlab®** – The package file containing the script can be imported using the Import tool in the Method Editor Program of the Hamilton® VENUS® software. The verified script will still need to be configured prior to use. Labware teaching, liquid class functionality, and any deck modifications should be checked. Please contact a Hamilton service engineer for instructions on how to import the package and perform calibration tests.

- ✓ **Tecan® Fluent®** - The package file containing the script can be imported using the Export/Import tool in the Tecan® FluentControl™ application.
  Instructions can be viewed at https://www.tecan.com/knowledge-portal/how-to-use-the-export/import-tool-in-fluentcontrol. The verified script will still need to be configured prior to use. Labware teaching, liquid class functionality, and any deck modifications should be checked. It is recommended to contact a Tecan® service engineer to import the package and perform calibration tests.

- ✓ **KingFisher™ Flex** – The script can be imported through the Thermo Scientific™ BindIt™ software.
  Instructions can be found on page 43 of the BindIt Software User Manual at https://assets.thermofisher.com/TFS-Assets/LSG/manuals/BindIt_4_KingFisherInstrumentsUserManual.pdf. Once imported, the script is ready to be used.

Pre-Run Preparation

The following steps are to be performed before every run.

- ✓ Check Appendix for best practices/tips.
- ✓ Use new reagents each run for optimal performance.

Buffer Preparation

1. Add 144 ml 100% ethanol (156 ml of 95% ethanol) to the 36 ml **M-Wash Buffer** concentrate.

2. Mix **EZ-Methylation Magprep Beads** well before use.

   Note: (OPTIONAL) If a large volume 96-channel pipetting head is available, the entire bottle of **EZ-Methylation Magprep Beads** can be directly added to the bottle of **M-Binding Buffer** before processing. Remove reagent distribution step of **EZ-Methylation Magprep Beads** and adjust **M-Binding Buffer** aliquot volume to 610 µl. Resuspend the slurry using pipette mixing prior to aliquoting into the binding plate. Contact automation@zymoresearch.com for support.

Conversion Reaction (Sample Preparation)

- ✓ Use **Lightning Conversion Reagent** immediately after opening.
- ✓ Set thermal cycler reaction volume to maximum if 150 µl is unavailable.
- ✓ Recommended to perform manually.

Note: The sample preparation steps can be automated on liquid handlers upon request.

1. Add 130 µl of **Lightning Conversion Reagent** to 20 µl of a DNA sample in a non-skirted 96-well microplate (user provided). Mix thoroughly by pipette mixing.

   Note: If the volume of DNA is less than 20 µl, compensate with water.

2. Seal the plate with a film (user provided) then briefly centrifuge. Transfer the samples to a thermal cycler and perform the following steps:

   1. 98 °C for 8 minutes
   2. 54 °C for 60 minutes
   3. 4 °C storage for up to 20 hours

   Note: The 4 °C storage step is *optional*.

Hamilton® Microlab® STAR™/STARlet™ Set-Up

A. Hardware & Labware Recommendations

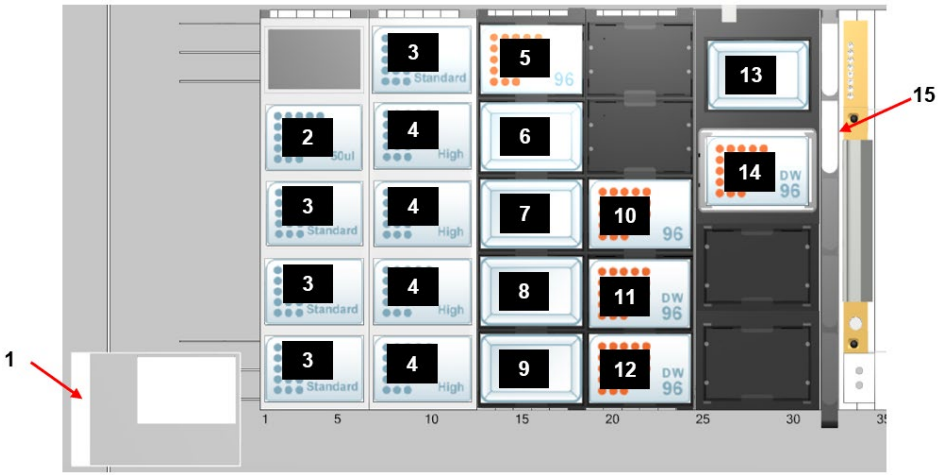
The STAR™ and STARlet™ have the same recommended hardware & labware.

Hardware Description	Source	Catalog
Magnum FLX® 96-Well	Alpaqua®	A000400
Hamilton® Heater Shaker, Flat Bottom Adapter	Hamilton®	199034
1000 µl 8-Channel CO-RE Head	Hamilton®	173081
1000 µl 96-Channel CO-RE Head	Hamilton®	199090
1mL CO-RE Gripper for Waste Block	Hamilton®	188066

Labware Description	Source	Catalog
Nunc™ 96-Well Polypropylene DeepWell™ Storage Plates 2ML	Thermo Scientific™	278743
Hard-Shell® Low-Profile, Thin-Wall, Skirted 96-Well PCR Plates	Bio-Rad®	HSP-9631
60 mL Reagent Reservoir Self-Standing	Hamilton®	194051
250 mL Reagent Reservoir 96-well, SLAS-ANSI	Hamilton®	56669-01
50 µL CO-RE Disposable Tips with Filters	Hamilton®	235979
300 µL CO-RE Disposable Tips with Filters	Hamilton®	235938
1000 µL CO-RE Disposable Tips with Filters	Hamilton®	235940

B. Deck Configuration

The following Hamilton® Microlab® STAR™ worktable is an example deck layout that is also compatible with the Microlab® STARlet™. The configuration can be remapped to fit custom deck layouts.



Position	Hardware/Labware Description
1	Tip Waste Chute
2	50 µL CO-RE Tips Disposable with Filters
3	300 µL CO-RE Disposable Tips with Filters
4	1000 µL CO-RE Disposable Tips with Filters
5	Elution Plate – Bio-Rad® Hard-Shell 96-Well PCR Plate
6	**M-Elution Buffer** – 300mL Trough
7	**L-Desulphonation Buffer** – 300mL Trough
8	**M-Wash Buffer** – 300mL Trough
9	**M-Binding Buffer** – 300mL Trough
10	Sample Plate – non-skirted 96-well PCR plate on adapter
11	Working Plate – Nunc™ DeepWell™ 2mL Plate
12	Magnum FLX® 96-Well
13	Waste Trough – 300mL Trough
14	Hamilton Heater Shaker
15	**EZ-Methylation Magprep Beads** – 60mL Trough

C. Microlab® STAR™/STARlet™ Run Instructions

- ✓ Set-Up can be performed during the conversion reaction.
- ✓ Resuspend **EZ-Methylation Magprep Beads** prior to loading into trough.

1. Follow the pre-run preparation instructions on page 7 to prepare the conversion reaction plate and **M-Wash Buffer**.
2. Initialize the instrument using the Hamilton® VENUS® software and start the script**.**
3. Follow the on-screen instructions to properly load reagents into correct troughs.
4. Place the reagent troughs and other labware into the deck as indicated from on-screen instructions.
5. Pause here if the conversion reaction is not completed.
6. Load the conversion reaction plate into the instrument as indicated from on-screen instructions.
7. Press “next” for the sample input prompt.
8. Input the number of samples being processed and desired elution volume.
9. Press “next” and the script will begin.
10. Remove elution plate from instrument once script is complete.

Tecan® Fluent® 480/720/1080 Set-Up

A. Fluent® 480 Hardware & Labware Recommendations

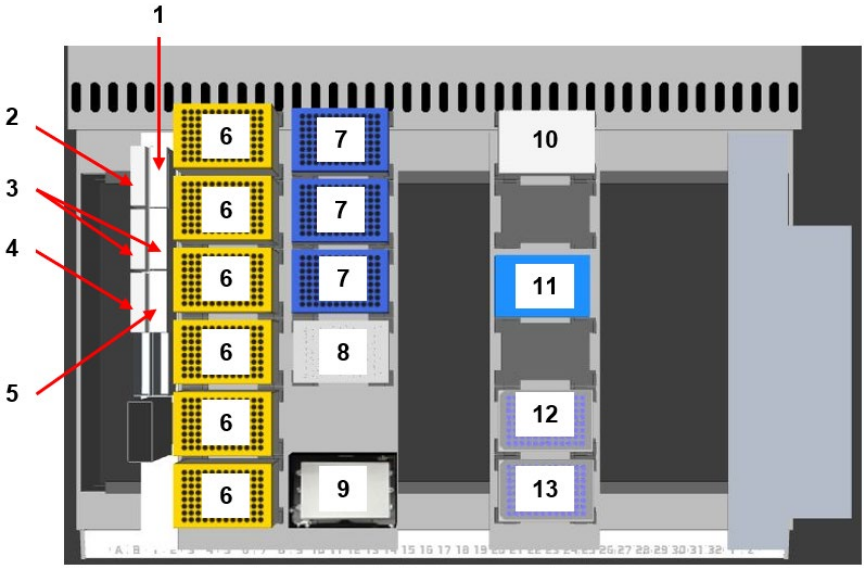
The Fluent® 480 has different hardware and labware recommendations than the 720/1080 due to size limitations (MCA not recommended).

Hardware Description	Source	Catalog
Magnum FLX® 96-Well	Alpaqua®	A000400
BioShake® D30-T ELM	Tecan®	30125516
Nunc 96 2mL Block Adapter Qinstruments®	Tecan®	30150449
Flexible Channel Arm™ Air (FCA)	Tecan®	30042200
Robotic Gripper Arm™	Tecan®	30042400

Labware Description	Source	Catalog
Nunc™ 96-Well Polypropylene DeepWell™ Storage Plates 2ML	Thermo Scientific™	278743
Hard-Shell® Low-Profile, Thin-Wall, Skirted 96-Well PCR Plates	Bio-Rad®	HSP-9631
DiTi LIHA 50µL CONDU.FIL. 2304 PCE. SBS	Tecan®	30057813
DiTi LIHA 200µL CONDU.FIL. 2304 PCE. SBS	Tecan®	30057815
DiTi LIHA 1000µL CONDU.FIL. 2304 PCE. SBS	Tecan®	30057817
LIHA DiTi SBS Box Refill Small 10 PCE.	Tecan®	30058506
LIHA DiTi SBS Box Refill Large 10 PCE.	Tecan®	30058507
Trough Disposable 25ML PP 120 PCE.	Tecan®	30055743
Trough Disposable 100ML PP Grey 108 PCE.	Tecan®	10613049
300mL Automation Friendly Reservoir Bases	Integra®	6305
300mL Automation Friendly Reservoir 100 bulk	Integra®	6328

B. Fluent® 480 Deck Configuration

The following Tecan® Fluent® 480 worktable is an example deck layout. The configuration can be remapped to fit custom deck layouts.



Position	Hardware/Labware Description
1	**EZ-Methylation Magprep Beads** – 25mL Trough
2	**M-Elution Buffer** – 100mL Trough
3	**M-Wash Buffer** – 2x100mL Troughs
4	**M-Binding Buffer** – 100mL Trough
5	**L-Desulphonation Buffer** – 100mL Trough
6	1000µL FCA DiTi Tips
7	200µL FCA DiTi Tips
8	Working Plate – Nunc™ DeepWell™ 2mL Plate
9	BioShake® D30-T ELM
10	Waste Trough – 300mL Trough
11	Magnum FLX® 96-Well
12	Elution Plate – Bio-Rad® Hard-Shell 96-Well PCR Plate
13	Sample Plate – non-skirted 96-well PCR plate on adapter

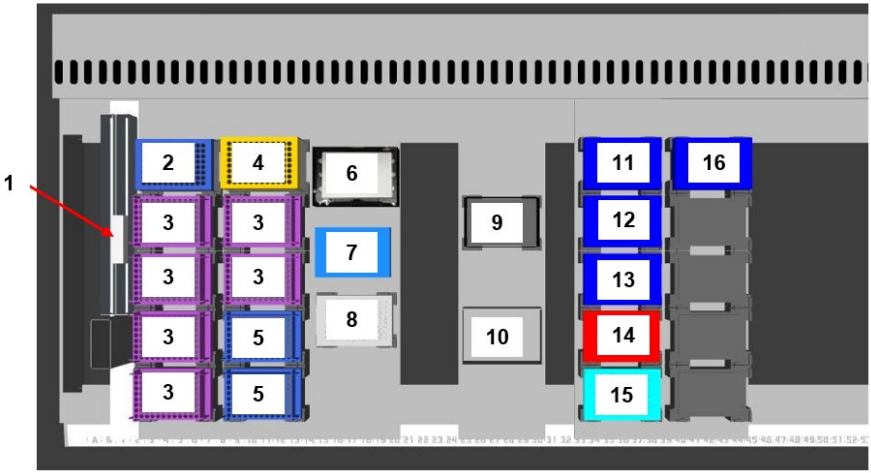
C. Fluent® 720/1080 Hardware & Labware Recommendations

Hardware Description	Source	Catalog
Magnum FLX® 96-Well	Alpaqua®	A000400
BioShake® D30-T ELM	Tecan®	30125516
Nunc 96 2mL Block Adapter Qinstruments®	Tecan®	30150449
Flexible Channel Arm™ Air (FCA)	Tecan®	30042200
Multiple Channel Arm™ 384/96	Tecan®	30042350
MCA 96 Format EVA Adapter DITI	Tecan®	30032062
Robotic Gripper Arm™	Tecan®	30042400

Labware Description	Source	Catalog
Nunc™ 96-Well Polypropylene DeepWell™ Storage Plates 2ML	Thermo Scientific™	278743
Hard-Shell® Low-Profile, Thin-Wall, Skirted 96-Well PCR Plates	Bio-Rad®	HSP-9631
DiTi LIHA 50µL CONDU.FIL. 2304 PCE. SBS	Tecan®	30057813
DiTi LIHA 200µL CONDU.FIL. 2304 PCE. SBS	Tecan®	30057815
DiTi LIHA 1000µL CONDU.FIL. 2304 PCE. SBS	Tecan®	30057817
LIHA DITI SBS Box Refill Small 10 PCE.	Tecan®	30058506
LIHA DITI SBS Box Refill Large 10 PCE.	Tecan®	30058507
Trough Disposable 25ML PP 120 PCE.	Tecan®	30055743
MCA 96 200 µL STERILE FIL. 3840 PCE. SBS	Tecan®	30038618
MCA 96 500 µL STERILE FIL. 3840 PCE.	Tecan®	30046342
300ML Automation Friendly Reservoir Bases	Integra®	6305
300ML Automation Friendly Reservoir 100 bulk	Integra®	6328

D. Fluent® 720/1080 Deck Configuration

The following Tecan® Fluent® 720 worktable is an example deck layout that is also compatible with the Fluent® 1080. The configuration can be remapped to fit custom deck layouts.



Position	Hardware/Labware Description
1	**EZ-Methylation Magprep Beads** – 25mL Trough
2	200µL FCA DiTi Tips
3	500µL MCA DiTi Tips
4	1000µL FCA DiTi Tips
5	150µL MCA DiTi Tips
6	BioShake® D30-T ELM
7	Magnum FLX® 96-Well
8	Working Plate – Nunc™ DeepWell™ 2mL Plate
9	Waste Trough – 300mL Trough
10	MCA Through Deck DiTi Waste Chute
11	**M-Binding Buffer** – 300mL Trough
12	**M-Wash Buffer** – 300mL Trough
13	**L-Desulphonation Buffer** – 300mL Trough
14	Elution Plate – Bio-Rad® Hard-Shell 96-Well PCR Plate
15	Sample Plate – non-skirted 96-well PCR plate on adapter
16	**M-Elution Buffer** – 300mL Trough

E. Tecan® Run Instructions

- ✓ Set-Up can be performed during the conversion reaction.
- ✓ Resuspend **EZ-Methylation Magprep Beads** prior to loading into trough.

1. Follow the pre-run preparation instructions on page 7 to prepare the conversion reaction plate and **M-Wash Buffer**.

2. Initialize the instrument using the Tecan® FluentControl™ application and start the script**.**

3. Follow the on-screen instructions to properly load reagents into correct troughs.

4. Place the reagent troughs and other labware into the deck as indicated from on-screen instructions.

5. Pause here if the conversion reaction is not completed.

6. Load the conversion reaction plate into the instrument as indicated from on-screen instructions.

7. Press “next” until the script main menu is reached.

8. Input the number of samples being processed and desired elution volume.

9. Press “next” and the script will begin.

10. Remove elution plate from instrument once script is complete.

Thermo Scientific™ KingFisher™ Flex Set-Up

A. Automated-Elution and Manual-Elution Script Differences

The Automated-Elution script will perform all the automated steps including drying and elution. When eluting in 25 µl of elution buffer using the Automated-Elution script, only 17-18 µl can be recovered due to bead retention. The Manual-Elution script will perform all the automated steps up until drying. Users will then manually transfer the beads to a 96-well microplate to dry and subsequently elute for higher recovery. Although the instrument run-time is shorter on the Manual-Elution script (0.5 hours) than the Automated-Elution script (1.2 hours), the total processing time is the same. Users who wish to maximize yield should use the Manual-Elution script.

B. Hardware & Labware Recommendations

The following is a list of hardware and labware recommendations for both KingFisher™ Flex scripts.

Hardware Description	Source	Catalog
96 Deep-Well Head for KingFisher™ Flex Magnetic Particle Purification System	Thermo Scientific™	24074430

Labware Description	Source	Catalog
KingFisher™ 96 deep-well plate, v-bottom, polypropylene	Thermo Scientific™	95040450
KingFisher™ 96 tip comb for deep-well magnets	Thermo Scientific™	97002534
KingFisher™ 96 microplate (200µL)	Thermo Scientific™	97002540

Additional Materials	Source	Catalog
96-Well Side Pull Bar Magnet PCR Plate	Permagen®	MSP750

C. KingFisher™ Flex Manual-Elution Run Instructions

- ✓ Set-Up can be performed during the conversion reaction.
- ✓ Prepare all plates before loading the instrument.
- ✓ Resuspend **EZ-Methylation Magprep Beads** prior to aliquoting.

1. Follow the pre-run preparation instructions on page 7 to prepare the conversion reaction plate and **M-Wash Buffer**.

2. Fill the KingFisher™ plates according to the following table:

Plate Name	Plate Type	Reagent/Tip Content	Reagent Volume
Tip Plate	96 DW Plate	96 Deep-Well Tip Comb	N/A
Binding Plate	96 DW Plate	EZ-Methylation Magprep Beads	10 µl
		M-Binding Buffer	600 µl
M-Wash 1	96 DW Plate	M-Wash Buffer	400 µl
L-Desulphonation	96 DW Plate	L-Desulphonation Buffer	200 µl
M-Wash 2	96 DW Plate	M-Wash Buffer	400 µl
Wash Elution	96 Standard Plate	M-Wash Buffer	150 µl

3. Transfer 150 µl of each sample from the conversion reaction plate into the binding plate.

4. Navigate to the script using the instrument display and press start.

5. Load the plates according to the displayed instructions.

6. Press start after the last plate is loaded and the script will begin.

7. Remove the wash elution plate containing **M-Wash Buffer** and **EZ-Methylation Magprep Beads** once the script is complete.

8. Resuspend and transfer 150 µl of each sample to a clean non-skirted 96-well PCR plate (user provided).

   Note: Carefully resuspend the beads in the wash buffer and transfer the slurry. Some beads will be retained in the wash elution plate. Volume may be less than 150 µl due to evaporation.

9. Place the non-skirted 96-well PCR plate onto a 96-well microplate magnet for 2 minutes.

10. With the plate still on the magnet, carefully aspirate and remove the supernatant without disturbing the beads.

    Note: Remove as much supernatant as possible to aid drying.

11. Let the plate dry at room temperature for 30-40 minutes until beads appear matte brown.

    Note: A plate heating element at 55 °C can be used to increase drying speed. Beads may flake if overdried using heat.

12. With the plate still on the magnet, add 25 µl of **M-Elution Buffer** to each well containing beads.

    Note: Beads may flake when adding **M-Elution Buffer** if plate is not on magnet.

13. Remove the plate from the magnet and pipette mix each well containing beads.

14. Let the plate stand at room temperature for 5 minutes.

15. Place the plate back onto the magnet for 2 minutes.

16. With the plate still on the magnet, transfer the supernatant to a clean 96-well PCR microplate (user provided).

D. KingFisher™ Flex Automated-Elution Run Instructions

- ✓ Set-Up can be performed during the conversion reaction.
- ✓ Prepare all plates before loading the instrument.
- ✓ Resuspend **EZ-Methylation Magprep Beads** prior to aliquoting.

1. Follow the pre-run preparation instructions on page 7 to prepare the conversion reaction plate and **M-Wash Buffer**.

2. Fill the KingFisher™ plates according to the following table:

Plate Name	Plate Type	Reagent/Tip Content	Reagent Volume
Binding Plate	96 DW Plate	EZ-Methylation Magprep Beads	10 µl
		M-Binding Buffer	600 µl
M-Wash 1	96 DW Plate	M-Wash Buffer	400 µl
L-Desulphonation	96 DW Plate	L-Desulphonation Buffer	200 µl
M-Wash 2	96 DW Plate	M-Wash Buffer	400 µl
M-Wash 3/Tip	96 DW Plate	M-Wash Buffer	400 µl
		96 Deep-Well Tip Comb	N/A
Elution	96 Standard Plate	M-Elution Buffer	25 µl

3. Transfer 150 µl of each sample from the conversion reaction plate into the binding plate.

4. Navigate to the script using the instrument display and press start.

5. Load the plates according to the displayed instructions.

6. Press start after the last plate is loaded and the script will begin.

7. Remove the elution plate once the script is complete.

   Note: EZ-Methylation Magprep Beads may still be present in the elution plate and will be removed using the plate magnet.

8. Place the elution plate on a plate magnet for 2 minutes.

9. With the plate still on the magnet, transfer the supernatant to a clean 96-well PCR microplate (user provided).

Detailed Automated Desulphonation & Clean-Up Steps

The following detailed script steps can be used as a guideline to create or adapt a script.

- ✓ Perform all plate shaking steps at 1,300 – 1,600 RPM (e.g., BioShake® D30, Tecan® Te-Shake™).
- ✓ Load appropriate labware and consumables according to the script.
- ✓ Multi-dispense when possible if using 8-channel pipette head.

1. Dispense 600 µl of **M-Binding Buffer** and 10 µl of **EZ-Methylation Magprep Beads** to each well of a new 96-well deep well plate (user provided).

   Note: Ensure **EZ-Methylation Magprep Beads** are fully re-suspended in the reservoir before adding. Pre-mixing before dispense distribution is recommended.

2. Transfer 150 µl of each sample from the conversion reaction plate into the deep well plate containing the **M-Binding Buffer** and **EZ-Methylation Magprep Beads**, then plate shake for 5 minutes at room temperature. Alternatively, pipette mix thoroughly and let plate stand for 5 minutes at room temperature.

   Note: Remove the cover film from the conversion plate prior to sample transfer.

3. Transfer the plate to a magnetic stand for 4 minutes or until beads pellet and supernatant is cleared. With the plate on the magnetic stand slowly aspirate and discard the supernatant.

4. Transfer the plate off the magnetic stand for this and each subsequent buffer addition. Dispense 400 µl of **M-Wash Buffer** to each well containing beads. Re-suspend the beads by plate shaking for 30 seconds or pipette mixing. Replace the plate on the magnetic stand for 2 minutes or until beads pellet. Slowly aspirate and discard the supernatant.

5. Dispense 200 µl of **L-Desulphonation Buffer** to each well containing beads. Re-suspend the beads by plate shaking for 30 seconds or pipette mixing. Let the plate stand at room temperature for a total of 15 minutes. After the incubation, replace the plate on the magnetic stand for 2 minutes or until beads pellet. Aspirate and discard the supernatant.

   Note: Take time for handling into account for the total incubation time. Adjust time as necessary to ensure no sample remains in the **L-Desulphonation Buffer** for more than 25 minutes.

6. Pre-heat a plate heating element to 55 °C.

   Note: Pre-heating can be performed any time prior to step 8. If no heating element is available, drying at room temperature can be performed instead. The same plate shaker used for mixing can be heated.

7. Dispense 400 µl of M-Wash Buffer to each well containing beads. Re-suspend the beads by plate shaking for 30 seconds or pipette mixing. Replace the plate on the magnetic stand for 2 minutes or until beads pellet. Slowly aspirate and discard supernatant. Repeat this wash step 1.

   Note: Remove as much buffer as possible after final wash to aid in drying the beads 8 Remove as much buffer as possible after final wash to aid in drying the beads.

8. Transfer the plate to a heating element at 55 °C for 20-30 minutes to remove residual **M-Wash Buffer** and dry the beads.

   Note: Beads will change in appearance from glossy black to dark brown when fully dry.

9. Dispense ≥ 25 µl of **M-Elution Buffer** directly to the dried beads. Re-suspend the beads by plate shaking for 30 seconds or pipette mixing. Replace the plate on the heating element at 55 °C for 4 minutes then transfer the plate to the magnetic stand for 2 minutes or until beads pellet. Transfer the supernatant to a clean 96-well microplate (user provided).

   Note: Single aspiration and dispense recommended. **M-Elution Buffer** may splash on sides of wells when multi-dispensing.

   Note: If beads are removed with the elution, slowly pipetting up and down one or two times will allow them to be pulled back to the magnet.

The bisulfite converted DNA is ready for immediate analysis or can be stored at or below -20 °C for later use. For long term storage, store at or below -70 °C.

Appendix

Appendix I: Best Practices/Tips

- ✓ **Lightning Conversion Reagent** should be immediately used after opening. Avoid re-using leftover conversion reagent for optimal performance. Thoroughly spin samples down after mixing. Droplets of reagent on sides of wells can cause precipitation to form.

- ✓ **M-Binding Buffer** is slightly more viscous than the other reagents. Slow aspiration is recommended when removing supernatant to prevent loss of beads. For large volume dispensing (96-channel head), **EZ-Methylation Magprep Beads** can be pre-mixed with **M-Binding Buffer** in a trough. Mix well and dispense 610 µl of the slurry into each well.

- ✓ **EZ-Methylation Magprep Beads** quickly settle in solution and may dispense unevenly across plate rows affecting consistency. Pre-mixing the beads and using a multi-dispense workflow will reduce inconsistencies (ex. aspirate 120uL and dispense 10uL into each column in a 96-Well plate). Beads will change in appearance from matte black to dark brown indicating dryness.

- ✓ **L-Desulphonation Buffer** should not remain in contact with the beads for more than 25 minutes. Incubating longer than 25 minutes may decrease converted DNA quality. Adjust the speed of the supernatant removal step as necessary. Multi-dispense when using 8-channel liquid handler recommended.

- ✓ **M-Wash Buffer** requires an addition of ethanol before use. Thorough drying of the beads is needed to prevent **M-Wash Buffer** from being carried downstream. Drying duration may vary based on the laboratory climate. Multi-dispense when using 8-channel liquid handler recommended.

- ✓ **M-Elution Buffer** may be retained by the **EZ-Methylation Magprep Beads** during the final elution transfer step. Slow aspiration at Z bottom of the plate is recommended. If the beads are removed with the elution buffer, slowly dispense and aspirate again to allow the beads to be pulled to the magnet. Multi-dispense is not recommended as dispensing low volumes at high Z-values can result in **M-Elution Buffer** splashing on sides of wells.

Frequently Asked Questions

Q: Should the input DNA be dissolved in TE, water, or some other buffer prior to its conversion?

A: *Water, TE or modified TE buffers can be used to dissolve the DNA and do not interfere with the conversion process.*

Q: The EZ-Methylation Magprep Beads are unevenly distributed across the plate. Will this affect conversion efficiency?

A: *The conversion efficiency should not be affected by the distribution. Only the yields and drying duration of different columns may be affected. We recommend thoroughly resuspending the beads before dispensing them.*

Q: What are the differences between pipette mixing and plate-shaking?

A: *Plate shaking allows for faster processing speeds. Both pipette mixing and plate shaking will provide >99.5% conversion efficiency.*

Q: Would using a 96-channel pipetting head increase processing speed?

A: *Yes, our liquid handler execution times assume the use of 8-channel heads. Using a 96-channel head will increase processing speed and can help to avoid over-incubation during the* ***L-Desulphonation Buffer*** *step.*

Q: The detailed guidelines are liquid handler specific. How do I implement this workflow onto my Kingfisher™ Flex myself?

A: *We provide two different verified Kingfisher™ Flex scripts that contain the Desulphonation & Clean-up steps (see page 16). These scripts can be used as guidelines by users to write their own scripts.*

Q: What is the main difference between the Automated-Elution and the Manual-Elution Kingfisher™ Flex scripts?

A: *The Automated-Elution script will perform all the automation steps including drying and elution while the Manual-Elution script stops before drying. The Manual-Elution script allows for higher recovery of the sample input but requires more hands-on time.*

Q: Which steps are recommended to use multi-dispensing?

A: *We recommend multi-dispensing when adding* ***M-Wash Buffer*** *and* ***L-Desulphonation Buffer****.*

Q: Can I create my own script instead?

A: *Yes, we provide a guideline for users to create their own script on pages 20-21 of the protocol.*

Q: Can the entire sample preparation be automated?

A: *Yes, all the conversion reaction (sample preparation) steps can be performed on liquid handlers with the use of a programmed on-deck thermal cycler. Users will need to purchase additional equipment and perform validation tests. Contact automation@zymoresearch.com for support.*

Ordering Information

Product Description	Catalog No.	Size
EZ DNA Methylation-Lightning™ Automation Kit	D5049	96 preps.

Individual Kit Components	Catalog No.	Amount
Lightning Conversion Reagent	D5030-1 D5032-1	1 tube 1 bottle
M-Binding Buffer	D5001-3 D5002-3 D5049-3 D5040-3	20 ml 80 ml 100 ml 250 ml
M-Wash Buffer	D5001-4 D5002-4 D5007-4 D5040-4	6 ml 24 ml 36 ml 72 ml
L-Desulphonation Buffer	D5030-5 D5031-5 D5046-5	10 ml 40 ml 80 ml
M-Elution Buffer	D5001-6 D5002-6 D5007-6 D5041-6 D5049-6	1 ml 4 ml 8 ml 40 ml 50 ml
EZ-Methylation Magprep Beads	D4100-5-4 D4100-5-8 D4100-5-16	4 ml 8 ml 16 ml

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Quick-DNA/RNA MagBead kit Cat. No. R2130, R2131	Total DNA and RNA from any sample.

✓ For automated, high-throughput rapid and complete bisulfite conversion of DNA:

Epigenetics – Bisulfite Conversion	
EZ-96 DNA Methylation MagPrep Cat. No. D5040, D5041	>99% Bisulfite conversion efficiency.
EZ-96 DNA Methylation-Lightning Automation Kit Cat. No. D5049	>99.5% Bisulfite conversion efficiency.

✓ For high-quality NGS methylation analysis:

Epigenetics – NGS	
Zymo-Seq WGBS Library Kit Cat. No. D5465	WGBS library preparation in less than 4 hours and in one tube.
Zymo-Seq RRBS Library Kit Cat. No. D5460, D5461	RRBS library preparation in 2 hours from ≥10ng input.
Human Methylation & Non-methylated DNA Set Cat. No. D5014	Positive and negative controls for methylation detection applications.

Notes

Notes

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