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Innovating Epigenetic Solutions

QUICK GUIDE

MicroPlex Library Preparation kit v2

For use with: MicroPlex Library Preparation kit v2 x12 (12 indices), Cat. No. C05010012
MicroPlex Library Preparation kit v2 x48 (12 indices), Cat. No. C05010013
MicroPlex Library Preparation kit v2 x48 (48 indices), Cat. No. C05010014

MicroPlex Library Preparation v2 builds on the innovative MicroPlex chemistry to generate DNA libraries with expanded multiplexing capability and with even greater performance. kits contain either 12 or 48 single read Illumina®-compatible indexes. MicroPlex v2 can be used in DNA-seq, RNA-seq, or ChIP-seq and offers robust target enrichment performance with all of the leading platforms.

For detailed protocol, **please refer to the MicroPlex Library Preparation kit v2 Instruction Manual** at www.diagenode.com.

Storage and Handling: Store kit at –20°C upon arrival. Prior to use, transfer enzymes to ice and centrifuge briefly. Thaw buffers, vortex briefly and centrifuge prior to use. Keep all enzymes and buffers on ice until used.

A. Kit contents

Name	Cap Color	12 rxns/ 48 rxns (12 indices)	48 rxns (48 indices)
Template Preparation Buffer	Red	1 Tube	1 Tube
Template Preparation Enzyme	Red	1 Tube	1 Tube
Library Synthesis Buffer	Yellow	1 Tube	1 Tube
Library Synthesis Enzyme	Yellow	1 Tube	1 Tube
Library Amplification Buffer	Green	1 Tube	1 Tube
Library Amplification Enzyme	Green	1 Tube	1 Tube
Nuclease-Free Water	Clear	1 Tube	1 Tube
Indexing Reagents	Blue	12 Tubes	1 Single Index P

B. Notes before starting

1. Input DNA sample requirements: please refer to the MicroPlex Library Preparation kit v2 Instruction Manual for detailed instructions on preparing DNA samples.

	Requirement
Nucleic acid	Fragmented double-stranded DNA or cDNA
Source	Cells, plasma, urine, other biofluids, FFPE tissues, fresh tissues, frozen tissues
Type	Mechanically sheared; enzymatically fragmented; low molecular weight cell-free DNA ChIP DNA;
Molecular weight	< 1000 bp
Input amount	50 pg – 50 ng
Input volume	10 µL
Input buffer	≤ 10 mM Tris, ≤ 0.1 mM EDTA

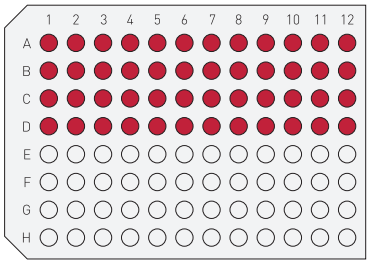
2. Additional materials and equipment needed: Thermal cycler with 50 µL reaction volume capability and heated lid; centrifuge; PCR tubes or plates; PCR plate seals; low binding barrier tips; fluorescent dyes; Agencourt® AMPure® XP (Beckman Coulter, CAT. NO. A63880), 80% v/v Ethanol.
3. Selecting PCR Plates/Tubes: Select plates/tubes that are compatible with the thermal cyclers and/or real-time PCR instruments used. Ensure that there is no evaporation during the process by using proper seal/caps during cycling as evaporation may reduce reproducibility.
4. Positive and Negative Controls: If necessary, include a positive control DNA (eg. Coriell DNA, sheared, 200 – 300 bp,) and a No Template Control (NTC) as a negative control in parallel to ensure that the reaction proceeded as expected.
5. Preparation of Master Mixes: Keep all enzymes and buffers on ice. Library Synthesis Master Mix and Library Amplification Master Mix can be prepared during the last 15 minutes of the previous step's cycling protocol and kept on ice until used.
6. Indexing Reagents: Indexing Reagents can be frozen and thawed no more than four times.
   - MicroPlex Library Preparation kit v2 x48 (48 indices) is provided with a Single Index Plate (SIP) containing 48 Illumina-compatible single indexes, each with a unique 8-nucleotide Sanger index sequence (see table below). The SIP is sealed with pierceable sealing foil; each well has sufficient volume for a single use.
   - MicroPlex Library Preparation kit v2 x12 (12 indices) are provided with 12 Indexing Reagents pre-dispensed in tubes. They have sufficient reagents for up to 8 uses and contain 8-nucleotide Sanger indexes (see table below) that share the same sequences in the first 6 bases as the Illumina TruSeq® LT indexes AD001 through AD012.
7. Single Index Plate (SIP) Handling Instructions: Follow the instructions given below to avoid index cross contamination.
   - Thaw the SIP for 10 min on the bench top prior to use. Once thawed, briefly centrifuge the plate to collect the contents to the bottom of each well. Thoroughly wipe the foil seal with 70% ethanol and allow it to dry completely.
   - Pierce the seal above each well containing the specific index combination with a clean 200 µL filtered pipet tip; discard the tip.
   - Use a new pipet tip to collect 5 µL of a specific index combination and add it to the reaction mixture at the Library Amplification Step. A multichannel pipette may be used if needed. If indexes from the entire plate are not used at the same time (MiSeq only, see section B.8 below), follow the instructions below to avoid contamination:
   - Cover any pierced index wells with scientific tape (such as VWR General Scientific Tape 0.5", CAT. NO. 89097-

920) to mark the index as used.

- Once the Index Plate is used, wipe the seal with 70% ethanol and let it dry completely. Replace the plastic lid and return the plate to its sleeve and store at -20°C.

8. Low level multiplexing: Select index combinations that meet the Illumina recommended compatibility requirements. For MicroPlex Library Preparation kit v2 x48 (48 indices) kit, multiplexing less than the full set of 48 libraries is possible on MiSeq only because MiSeq RTA v1.17.28 and later can process low-plexity index reads. For more information on multiplexing and index pooling, please refer to Appendix 1 of the MicroPlex Library Preparation kit v2 Instruction Manual at www.diagenode.com

9. Index Plate Maps:



Single Index Plate (48 rxns, 48 indices)

Well	Sequence	Well	Sequence	Well	Sequence	Well	Sequence
A1	ATCACGTT	B1	TGGTTGTT	C1	TGCGATCT	D1	GGCACAAC
A2	CGATGTTT	B2	TCTCGGTT	C2	TTCCTGCT	D2	TCTCACGG
A3	TTAGGCAT	B3	TAAGCGTT	C3	TAGTGACT	D3	TCAGGAGG
A4	TGACCACT	B4	TCCGTCTT	C4	TACAGGAT	D4	TAAGTTCG
A5	ACAGTGGT	B5	TGTACCTT	C5	TCCTCAAT	D5	TCCAGTCG
A6	GCCAATGT	B6	TTCTGTGT	C6	TGTGGTTG	D6	TGTATGCG
A7	CAGATCTG	B7	TCTGCTGT	C7	TAGTCTTG	D7	TCATTGAG
A8	ACTTGATG	B8	TTGGAGGT	C8	TTCCATTG	D8	TGGCTCAG
A9	GATCAGCG	B9	TCGAGCGT	C9	TCGAAGTG	D9	TATGCCAG
A10	TAGCTTGT	B10	TGATACGT	C10	TAACGCTG	D10	TCAGATTC
A11	GGCTACAG	B11	GTGCTACC	C11	TTGGTATG	D11	TACTAGTC
A12	CTTGTACT	B12	GGTTGGAC	C12	TGAACTGG	D12	TTCAGCTC

C. Quick protocol

I. Template Preparation Step

1. Add 10 µL of DNA sample to each well of a PCR plate or tube. If necessary, include NTC negative control buffer sample(s) and positive control samples.
2. Depending on the number of reactions, prepare the Template Preparation Master Mix as described in the table below. Mix thoroughly with a pipette. Keep on ice until used.

Template Preparation Master Mix		
Component	Cap Color	Volume/Reaction
Template Preparation Buffer	Red	2 µL
Template Preparation Enzyme	Red	1 µL

3. To each 10 µL sample from step 1 above, add 3 µL of the Template Preparation Master Mix.
4. Mix thoroughly with a pipette.

   Note: Final volume at this stage will be 13 µL.
5. Seal the PCR plate using proper sealing film or tightly cap the tube(s).
6. Centrifuge briefly to collect contents to the bottom of each well or tube.
7. Place the plate or tube(s) in a thermal cycler with a heated lid set to 101°C – 105°C. Perform the Template Preparation Reaction using the conditions in the table below.

Template Preparation Reaction	
Temperature	Time
22°C	25 min
55°C	20 min
4°C	Hold ≤ 2 hours

8. Remove the plate or tube(s) from the thermal cycler and centrifuge briefly.
9. Continue to the Library Synthesis Step.

II. Library Synthesis Step

1. Prepare Library Synthesis Master Mix as described in the table below. Mix thoroughly with a pipette. Keep on ice until used.

Template Preparation Master Mix		
Component	Cap Color	Volume/Reaction
Library Synthesis Buffer	Yellow	1 µL
Library Synthesis Enzyme	Yellow	1 µL

2. Remove the seal on the plate or open the tube(s).
3. Add 2 µL of the Library Synthesis Master Mix to each well or tube.
4. Mix thoroughly with a pipette.

   Note: Final volume at this stage is 15 µL.
5. Seal the PCR plate using proper sealing film or tightly cap the tube(s).
6. Centrifuge briefly to collect contents to the bottom of each well or tube.
7. Return the plate or tube(s) to the thermal cycler with a heated lid set to 101°C – 105°C. Perform Library Synthesis Reaction using the conditions in the table below.

Library Synthesis Reaction	
Temperature	Time
22°C	40 min
4°C	Hold ≤ 30 min

8. Remove the plate or tube(s) from the thermal cycler and centrifuge briefly.
9. Continue to the Library Amplification Step.

III. Library Amplification Step

1. Remove the Indexing Reagents from the freezer and thaw for 10 min on bench top. Prior to use, centrifuge the Indexing Reagents to collect the contents at the bottom. For 48S kit, wipe SIP foil seal with 70% ethanol and allow to dry.
2. Prepare Library Amplification Master Mix as described in the table below. Mix thoroughly with a pipette. Keep on ice until used.

Library Amplification Master Mix		
Component	Cap Color	Volume/Reaction
Library Amplification Buffer	Green	25.0 µL
Library Amplification Enzyme	Green	1.0 µL
Nuclease Free Water (plus fluorescent dyes*)	Clear	4.0 µL

* Fluorescence dyes (for detection and optical

calibration) are added when monitoring amplification in real time during cycling. Please refer to the Real Time PCR Instrument's user manual for calibration dye recommendations. The volume of detection and calibration dyes plus nuclease free water should not exceed 4 µL. If a regular thermal cycler is used, there is no need to add the dyes; use 4 µL of nuclease free water.

* Example: EvaGreen®/Fluorescein dye mix. Prepare by mixing 9:1 v/v ratio of EvaGreen Dye, 20X in water (Biotium, CAT. NO. 31000-T) and 1:500 diluted Fluorescein Calibration Dye (Bio-Rad Laboratories, CAT. NO. 170-8780); add 2.5 µL of this mix and 1.5 µL of nuclease free water per reaction.

3. Remove the seal on the PCR plate or open the tube(s).
4. Add 30 µL of Library Amplification Master Mix to each well or tube.
5. Add 5 µL of the appropriate Indexing Reagent to each well or tube.

   Note: For 48 rxns (48 indices) kit, follow the SIP handling instructions (section B.7 of this quick protocol) to avoid index cross contamination.

6. Mix thoroughly with a pipette. Avoid introducing excessive air bubbles.

   Note: Final volume at this stage is 50 µL.

7. Seal the plate or tube(s) tightly and centrifuge briefly to collect contents to the bottom of each well or tube.
8. Return plate or tube(s) to the real time PCR thermal cycler/ thermal cycler with a heated lid set to 101°C – 105°C. Perform Library Amplification Reaction using the cycling conditions from the tables below.

   Caution: Ensure that the thermal cycler does not have a denaturing step programmed until Stage 3.

Library Amplification Reaction				
	Stage	T°	Time	Number of Cycles
Extension & Cleavage	1	72°C	3 min	1
	2	85°C	2 min	1
Denaturation	3	98°C	2 min	1
Addition of Indexes	4	98°C	20 s	4
		67°C	20 s	
		72°C	40 s	
Library Amplification	5	98°C	20 s	5 to16 see table below
		*72°C	50 s	
	6	4°C	Hold	1
*Acquire fluorescence data at this step, if monitoring in real-time.				

* Selecting the optimal number of amplification cycles: The number of PCR cycles required at Stage 5 of the Library Amplification Reaction is dependent upon the amount of input DNA and the thermal cycler used. We recommend performing an optimization experiment to identify the appropriate number of PCR cycles needed. The table below provides the suggested number of PCR cycles at Stage 5 for different input amounts.

Stage 5 Amplification Guide	
DNA Input (ng)	Number of Cycles
50	5
20	6
10	7
5	8
2	10
1	11
0.2	14
0.05	16

Note: Over amplification could result in a higher rate of PCR duplicates in the library.

9. Remove the plate or tube(s) from the thermal cycler and centrifuge briefly.

   Note: At this stage, samples can be processed for next generation sequencing (NGS) immediately or stored frozen at –20°C and processed later. For instructions and recommendations on library pooling, purification, quantification, and sequencing, please refer to the MicroPlex Library Preparation v2 kit Instruction Manual at www.diagenode.com.

The 8nt index sequences were developed by the Wellcome Trust Sanger Institute in Cambridge, UK; additional information can be found in *Nature Methods* 7, 111 - 118 (2010). Illumina® is a registered trademark of Illumina, Inc.

RUBICON GENOMICS

MicroPlex Library Preparation Kit v2 x12 (12 indices) and MicroPlex Library Preparation Kit x48 (48 indices) contains ThruPLEX technology developed and manufactured by Rubicon Genomics, Inc., Ann Arbor, Michigan, USA and covered by US Patent 7,803,550; EP1924704; and US and international patents pending.

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