ZYMO RESEARCH

THE Epigenetics COMPANY™

Zymo-Seq ATAC Library Kit

Quickest, all-in-one ATAC-seq library preparation solution.

Highlights

- **Ready to Use:** Preassembled buffers allows for lightning-fast library preparation in as little as 4 hours without compromising quality.
- **Improved Performance:** Prepare libraries with 7x less mitochondrial contamination, saving reads and increasing sequencing depth.
- **Outstanding Consistency:** Produce highly correlated replicates from both fresh and frozen samples.

Catalog Numbers:
D5458

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

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Table of Contents

Product Contents

Zymo-Seq ATAC Library Kit	D5458 (12 reactions)	Storage Temperature
ATAC-S Buffer	300 µL	4 °C
ATAC Lysis Buffer	300 µL	-20 °C
ATAC Wash Buffer	12 mL	-20 °C
Tn5 Enzyme	35 µL	-20 °C
Pre-Tagmentation Buffer	600 µL	-20 °C
ATAC Library PCR Mix	300 µL	-20 °C
UDI Tag Primer Set (Indexes 1-12)	12 x 15 µL	-20 °C
Low Binding Tubes	12 tubes	Room Temperature
DNA Binding Buffer	25 mL	Room Temperature
DNA Wash Buffer	6 mL	Room Temperature
DNA Elution Buffer	1 mL	Room Temperature
Zymo-Spin IC Columns	25 tubes	Room Temperature
Collection Tubes	25 tubes	Room Temperature

Specifications

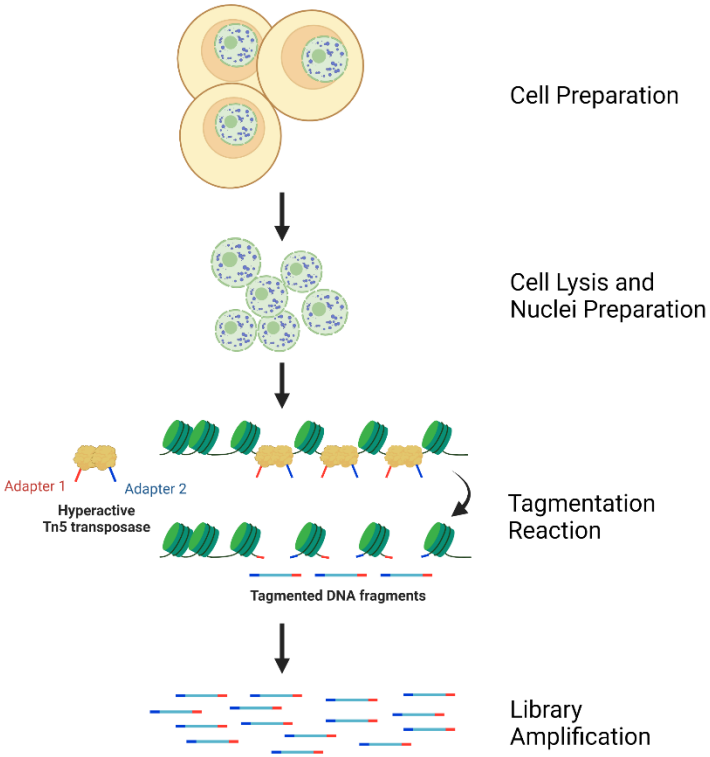
- **DNA Input:** For optimal results, use 50,000 cells per prep. Cells should present >80% viability (measure of the proportion of live cells within a population). It is recommended to use cell counter to measure viability accurately.
- **Equipment Required:** Microcentrifuge, biosafety cabinet, cell counter, water bath, thermocycler, and thermoshaker with capacity to heat to 37 °C and shake at 1000 rpm (We recommend a thermoshaker with a thermo block that can fit 1.5 mL tubes and 2mL Low Binding Tubes).
- **Processing Time:** 4 hours.
- **Library Storage:** Libraries eluted in **DNA Elution Buffer** may be stored at 4 °C overnight or at -20 °C for long term storage.
- **Sequencing Platform Compatibility:** Libraries are compatible with Illumina sequencing platforms (HiSeq, NextSeq, NovaSeq).
- **Reagents Not Provided:** Trypan blue, PBS, DNase I, cell media, trypsin, FBS, and DMSO.

Product Description

The Assay for Transposase-Accessible Chromatin with next generation sequencing (ATAC-Seq) is a convenient and powerful method to directly introduce sequencing adapter tags by high efficiency transposition. These adapter tags are inserted into unoccupied native chromatin sites within isolated nuclei from cells or tissue. The process known as tagmentation creates adapter tagged DNA fragments of open chromatin regions ready for final amplification and then sequencing. Open chromatin regions (sequenced regions) are typically enriched in promoter sites flanking actively transcribed genes while inaccessible regions (poorly sequenced regions) are occupied by nucleosomes or transcription factors. Therefore, determination of both open chromatin and inaccessible regions are directly linked to epigenetic gene regulation and chromatin states and structure, making ATAC-Seq an extremely valuable tool for vast functional epigenetic studies in cells and organisms.

Zymo-Seq ATAC Library Kit is an all-in-one, cost-effective ATAC-seq kit. Zymo offers exceptionally stable, premixed buffers that simplify the assay and cut down on hands-on time. With sample manipulation kept to a minimum, ready-to-sequence libraries can be generated in as little as 4 hours, and replicate reproducibility (Pearson's correlation > 0.95) is dramatically increased for both fresh and frozen samples**.** The simple streamlined workflow from cells to NGS libraries includes: (1) gentle cell lysis and nuclei prep; (2) tagmentation of open chromatin regions; (3) Index PCR amplification adds unique dual indexes to the library. See next page for a detailed **Zymo-Seq ATAC Library Kit** workflow.

Zymo-Seq ATAC Library Kit Workflow



Protocol

Before Starting:

- ✓ Ensure that thermoshaker is on and preheated to 37 °C.
- ✓ Thaw **ATAC Lysis Buffer** and **ATAC Wash Buffer,** then place on ice. Gently vortex prior to use.
- ✓ Add 24 mL of 100% ethanol (26 mL of 95% ethanol) to the 6 mL **DNA Wash Buffer** concentrate.
- ✓ Count cells with cell counter and determine viability. *Note: If viability is below 70%, see Appendix A3. on page 12.*
- ✓ Refer to **Appendix A: Cell Culture** for cell culture suggestions.

Section 1: Cell Lysis and Nuclei Preparation

1. Transfer 50,000 live cells per technical replicate to a nuclease free 1.5 mL tube.

2. Centrifuge cells at 300 x g for 4 minutes at room temperature.

3. Using a pipette, remove media without disturbing cell pellet. Resuspend cell pellet in 25 µL **ATAC-S Buffer** per technical replicate**.**

4. Aliquot 25 µL of the cell suspension into a labeled **Low Binding Tube** per technical replicate.
   *Example: To achieve four replicates, 200,000 live cells suspended in 100 µL **ATAC-S Buffer** are needed. The 100 µL cell suspension can then be aliquoted into four 25 µL replicates.*

5. Add 25 µL cold **ATAC Lysis Buffer** to each tube and mix by pipetting up and down 3 times.
   Note: Some bubbles are fine and will not affect the assay. We recommend a P200 pipette tip for mixing.

6. Incubate on ice for 3 minutes.

7. Add 1 mL of cold **ATAC Wash Buffer** to each of the tubes.
   *Note: Follow the same order as the **ATAC Lysis Buffer** was added.*

8. Invert the tube 5 times to mix.

Important: Place tubes in the microcentrifuge with hinges pointed towards the center of the rotor.

9. Centrifuge at 1,000 x g for 9 minutes at room temperature to pellet the nuclei.
   Note: The pelleted nuclei are extremely hard to see. It should be located away and opposite of the hinge, just off the bottom of the tube. If tube is agitated too much post centrifugation, the nuclei pellet may slide towards bottom of the tube.

10. During centrifugation in Step 9:
    Transposition Mix: Combine the following components on ice per reaction.

Components	Volume
Pre-Tagmentation Buffer	50 µL
Tn5 enzyme	2.75 µL
Total	52.75 µL

Section 2: Library Preparation

2.1 Tagmentation Reaction

1. Immediately after centrifugation, carefully remove the supernatant without disturbing the nuclei pellet. Note the following:
    a. The nuclei pellet may not be visible.
    b. If the supernatant is not removed immediately, the pellet may loosen and fall to the bottom of the tube. If removed quickly, the pipette tip can be placed directly at the bottom of the tube to remove supernatant.
    c. Any remaining supernatant will dilute the tagmentation reaction and will affect the assay performance.

2. Add 50 µL of **Transposition Mix** and gently resuspend the pellet by pipetting.

3. Incubate the reaction at 37 °C for 30 minutes in a thermoshaker set to 1,000 rpm. After incubation is complete, centrifuge tubes briefly to bring down any condensation.

4. Add 250 µL of **DNA Binding Buffer**. Vortex to mix and centrifuge tubes briefly to collect entire sample.

5. Transfer the sample to a pre-labeled **Zymo-Spin IC™ Column** in a **Collection Tube**.

6. Centrifuge at 16,000 x *g* for 30 seconds. Discard flow through.

7. Add 450 µL **DNA Wash Buffer**.

8. Centrifuge at 16,000 x *g* for 1 minute.

9. Carefully transfer **Zymo-Spin IC™ Column** to labeled 1.5 mL microcentrifuge tube.

10. Add 21 µL of **DNA Elution Buffer** directly to the column matrix. Let incubate at room temperature for 1 minute.

11. Centrifuge at 16,000 x *g* for 30 seconds to elute purified DNA. DNA can be stored at -20 °C until ready for library amplification.

2.2 Library Amplification

1. Thaw on ice:
    a. **ATAC Library PCR Mix**
    b. 25 uM **UDI Tag Primer(s)**
    c. 20 µL Tagmented DNA

2. Mix components in 0.2 mL PCR tube as follows:

Components	Volume
ATAC Library PCR Mix	25 µL
25 uM UDI Tag Primer	5 µL
Tagmented DNA	20 µL
Total	50 µL

Note: If replicates are to be sequenced together, be sure to use a different UDI primer in each replicate.

3. Place reaction mix into thermocycler and run following protocol (heated lid set to >100 °C):

Step	Temperature	Time
1	**72 °C**	**5 minutes**
2	**98 °C**	**30 seconds**
3	**98 °C**	**10 seconds**
4	**63 °C**	**30 seconds**
5	**72 °C**	**30 seconds**
6	**Repeat Steps 3-5 for a total of 10 cycles for cell lines.**	**-**
7	**4 °C**	**Hold**

Note: If using tissue samples, the number of cycles in step 6 of PCR will need to be optimized.

4. Transfer the reactions to 1.5 mL microcentrifuge tubes and add 250 µL **DNA Binding Buffer**. Vortex to mix and centrifuge briefly.

5. Transfer entire sample volume to pre-labeled **Zymo-Spin IC™ Column** in a **Collection Tube**.

6. Centrifuge at 16,000 x *g* for 30 seconds. Discard flow through.

7. Add 450 µL **DNA Wash Buffer**.

8. Centrifuge at 16,000 x *g* for 1 minute.

9. Carefully transfer **Zymo-Spin IC™ Column** to labeled 1.5 mL microcentrifuge tube.

10. Add 21 µL of **DNA Elution Buffer** directly to the column matrix. Let incubate at room temperature for 1 minute.

11. Centrifuge at 16,000 x g for 30 seconds to elute purified DNA.

12. Purified libraries are now ready for sequencing.

Appendices

Appendix A: Cell Culture

A1. Cell Preparation (start inside Biosafety Cabinet):

*Note: You need 50,000 total **live** cells per replicate.*

From fresh culture:

1. If using adherent cells, wash cell monolayer with PBS twice and then add Trypsin to dislodge cells from surface.
   Note: Follow standard cell culture SOP. Optimize trypsin conditions if needed.

2. Stop trypsinization with 2 volumes of complete media.
   Example: If cell media volume is 5 mL, use 10 mL complete media to stop trypsinization.

3. Thoroughly create a cell suspension by pipetting up and down using a serological pipette.

4. In a 1.5mL tube, mix 10 µL of homogenized cell culture with 10 µL of Trypan blue. Pipette up and down 10 times to ensure mixture is homogeneous.

5. Count cells with cell counter and determine viability.
   Note: If viability is below 70%, see Appendix A3 on page 12.

6. Transfer 50,000 live cells per technical replicate to a nuclease free 1.5 mL tube.
   Note: Cells can now be removed from Biosafety Cabinet.

7. Centrifuge cells at 300 x *g* for 4 minutes at room temperature.

8. Using a pipette, remove media without disturbing cell pellet. Resuspend cell pellet in 25 µL **ATAC-S Buffer** per technical replicate**.** Proceed to Section 1: Cell Lysis and Nuclei Preparation Step 4.

From cryopreserved cells:

1. Add 9 mL complete culture media to a 15 mL conical tube and bring up to 37 °C in a water bath.

2. Quickly thaw cryopreserved cells by gently swirling the cryovial in a 37 °C water bath until there is no more ice remaining in the vial.

3. Still within the biosafety cabinet, slowly pipette the thawed contents of the cryovial into the 9 mL of pre-warmed complete media.

4. Centrifuge cells at 500 x *g* for 4 minutes at room temperature.

5. Aspirate and discard media without disturbing the cell pellet, then resuspend cell pellet in 1 mL cold complete media using a 1mL pipette tip.

6. In a 1.5 mL tube, mix 10 µL of homogenized cell culture with 10 µL of Trypan blue. Pipette up and down 10 times to ensure mixture is homogeneous.

7. Count cells with cell counter and determine viability.
   Note: If viability is below 70%, see Appendix A3. on page 12.

8. Transfer 50,000 cells per technical replicate to a nuclease free 1.5mL tube.
   Note: Cells can now be removed from Biosafety Cabinet.

9. Centrifuge cells at 300 x *g* for 4 minutes at room temperature.

10. Using a pipette, remove media without disturbing cell pellet and resuspend cell pellet in 25 µL **ATAC-S Buffer** per technical replicate**.** Proceed to Section 1: Cell Lysis and Nuclei Preparation Step 4.

A2. Cell Counting:

We suggest using an automatic cell counting instrument for this purpose. If an automatic cell counter is not available, a manual protocol may be followed, and users should refer to the appropriate manual.

1. Prepare a mix of Trypan blue with cell suspension according to manufacturer's instructions for the cell counting instrument used.

2. Count viable cells (not stained by Trypan blue) and dead cells. Add both values together to get the number of total cells.

3. Determine the percent of viable cells for the cell suspension. (%viable = (#viable cells/ #total cells) x 100)

4. If percent of viable cells is below 70%, see Appendix section A3 for options.

5. Follow section steps and then recount cells to ensure high viability before proceeding in protocol.

A3. Cell viability recovery

Consider passaging cell culture and waiting 1-2 days before beginning protocol. Cells in fresh media should present better viability.

If cells cannot be passaged, treat cells with DNase following the protocol below.

DNase treatment for cell viability <70%:

1. Reconstitute DNase I to 20 U/µL in PBS.
    a. Centrifuge a 250U or 1500U tube of **Zymo DNase I**[1] at max speed for 5 minutes to pull the lyophilized DNase I to the bottom of the tube.
    b. To 250U **Zymo DNase I**, add 13.75 µL PBS.
    c. To 1500U **Zymo DNase I**, add 82.5 µL PBS.

2. Centrifuge the cells at 300 x *g* for 4 minutes at room temp.

3. Discard supernatant.

4. Resuspend cell pellet in 990 µL of PBS.

5. Add 10 µL of DNase I (20 U/µL) for each 990 µL of cell suspension (final conc. of 200 U/mL).

6. Incubate cells for 15 minutes at room temperature.

7. After incubation, spin cells at 300 x g for 4 minutes at room temp.

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[1] **DNase I Set** (Cat. E1010) is recommended for DNase treatment of cells.

8. Remove supernatant and resuspend cells in 1mL PBS.

9. Repeat steps 7 and 8 (spin and wash steps) to remove all DNase I.

10. After resuspension in PBS, count cells with cell counter and determine viability.

11. Transfer 300,000 cells to a fresh tube and add 1 mL PBS.

12. Spin cells at 300 x g for 4 minutes at room temperature.

13. Discard supernatant, resuspend cells in 150 µL **ATAC-S Buffer** and continue with protocol at **Section 1: Cell Lysis and Nuclei Preparation Step 4.**

*Note: If viability is extremely low (<50%), a majority of the cells undergoing tagmentation will be dead. For example, if viability is 10%, you're adding 300,000*0.9 = 270,000 dead cells along with those live cells. There is currently no procedure for removing the dead cells. Continue as if only live cells are present. Low viability can negatively affect sequencing noise.*

A4. Cryopreservation of cells:

Cryopreservation of cells can be performed using a standard protocol and appropriate freezing media to the cells being preserved. For cells lines, please consult the cell line provider to obtain a copy of the protocol used for cryopreservation. For primary cells, cryopreservation protocols should be widely available from published methods. As a general indication, cryopreservation media recipes typically include the same rich media used for cell culture (such as DMEM or RPMI), supplemented with 10-15% FBS and 5% DMSO. This general recipe is merely a guide and does not replace the need to find an actual cryopreservation recipe that works specifically for the cells being stored.

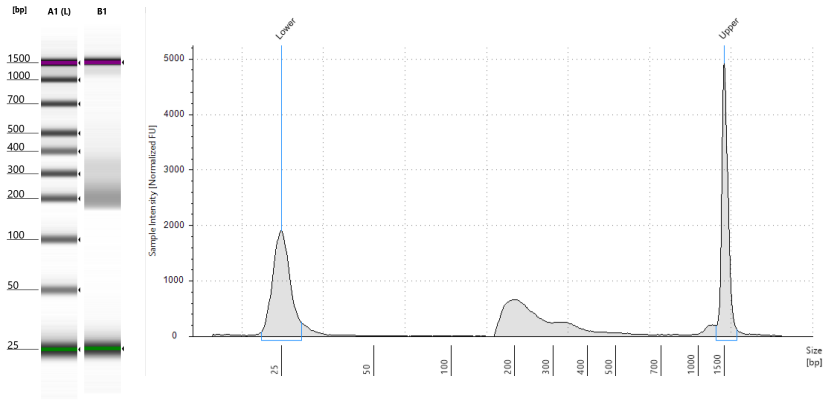
Appendix B: Library Validation and Quantification

Libraries should be visualized (i.e. agarose gel, Agilent TapeStation, Agilent Bioanalyzer, etc.) to determine that the correct library size is present, and no adapter dimers have formed. If dimers are present, they will form a ~140 bp band. Libraries ranging from approximately 200-700bp are ideal for sequencing. Below is an example of a completed Zymo-Seq ATAC library visualized of the Agilent TapeStation.

Libraries can be quantified using a preferred method (i.e. Nanodrop, Qubit, TapeStation, etc.). However, quantitative PCR is the recommended method for accurately determining library concentration prior to loading on to the Illumina sequencers.

Check Libraries on TapeStation:

- ✓ Run the libraries on TapeStation using the D1000 tape. (HSD1000 tape can be used if yield is lower than expected)



Characterization of ATAC-Seq libraries. A library was prepared with 50,000 cells from the human cell line HeLa, using the Zymo-Seq ATAC Library Kit and analyzed with Agilent TapeStation 2200. The library preparation produces approximately 169 – 700 bp fragments as shown on the D1000 Screen Tape gel (left) and electropherogram (right), with noticeable peaks around 200 and 335 bp. **A1** is a molecular weight marker and **B1** is ATAC-Seq library.

Appendix C: Considerations for Sequencing

Note that library pools can be immediately quantified after PCR or be frozen and stored at -20 °C until ready.

Preparation for clustering:

Accurate determination of the final library concentration is critical to achieve optimal clustering and sequencing results. For this, we recommend using quantitative PCR (e.g. KAPA Library Quantification Kit).

Sequencing parameters:

We recommend 50-bp paired-end sequencing with 60M reads per library for open chromatin regions or 230M reads per library for footprinting analysis. This will increase the likelihood of obtaining at least 50M or 200M non-mitochondrial, non-duplicate and high mapping quality reads for peak calling and footprint analysis, respectively.

Libraries are compatible with Illumina sequencing platforms (HiSeq, NextSeq, NovaSeq).

We recommend following the NF-Core/ATAC-seq pipeline for data analysis. For more information visit https://github.com/nf-core/atacseq

Appendix D: Unique Dual Index Primer Sequences

The kit supplies twelve (12) **UDI Tag Primer Sets**, each with unique i5 and i7 index sequences pooled together.

If multiplexing samples together, use a primer set only once per lane. To ensure color balance during multiplexing, use the Index Tag Primer sequentially (Ex: If pooling 2 samples, use Sets #1-2. If pooling 4 samples, use Sets #1-4.).

UDI Tag Primer Set	i7 Sequence for Sample Sheet	i5 Sequence for Sample Sheet
1	GAACTGAGCG	CGCTCCACGA
2	AGGTCAGATA	TATCTTGTAG
3	CGTCTCATAT	AGCTACTATA
4	ATTCCATAAG	CCACCAGGCA
5	GACGAGATTA	AGGATAATGT
6	AACATCGCGC	ACAAGTGGAC
7	CTAGTGCTCT	TACTGTTCCA
8	GATCAAGGCA	ATTAACAAGG
9	GACTGAGTAG	CACTATCAAC
10	AGTCAGACGA	TGTCGCTGGT
11	CCGTATGTTC	ACAGTGTATG
12	GAGTCATAGG	AGCGCCACAC

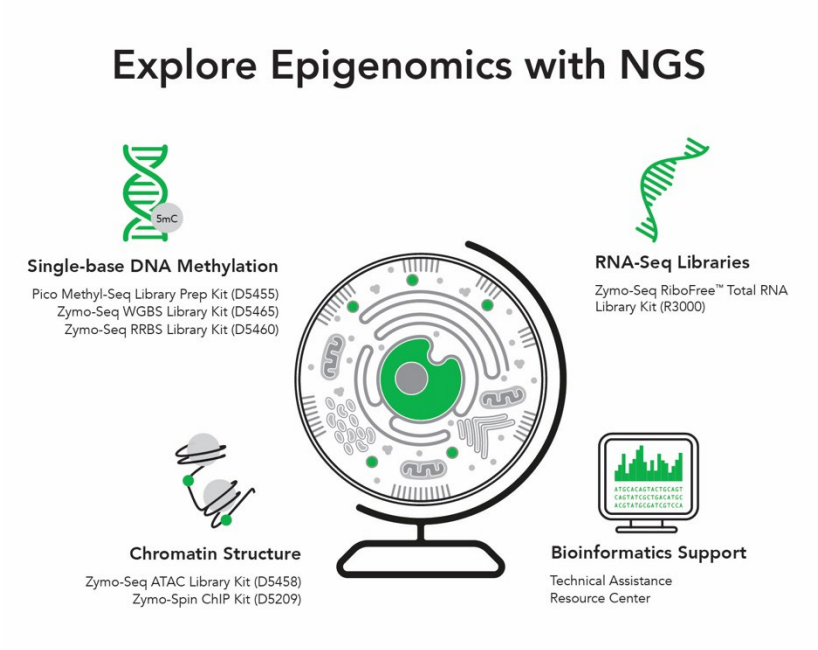
Ordering Information

Product Description	Catalog No.	Size
Zymo-Seq ATAC Library Kit	D5458	12 preps

Individual Kit Components	Catalog No.	Amount
DNA Binding Buffer	D4003-1-25 D4003-1-L D4004-1-L	25 ml 50 ml 100 ml
DNA Wash Buffer	D4003-2-6 D4003-2-24 D4003-2-48	6 ml 24 ml 48 ml
DNA Elution Buffer	D3004-4-1 D3004-4-4 D3004-4-10 D3004-4-16 D3004-4-50	1 ml 4 ml 10 ml 16 ml 50 ml
Zymo-Spin IC Columns	C1004-50 C1004-250	50 tubes 250 tubes
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 tubes 500 tubes 1000 tubes

Complete Your Workflow

Explore the complete Epigenome of your organism with Zymo-Seq NGS solutions. Obtain multi-omics overview by combining chromatin structure analysis with DNA methylation status and RNA expression. The Zymo-Seq portfolio provides a complete solution for all your transcription regulation studies, including comprehensive bioinformatic solutions for your analysis needs.



Notes

Notes

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Integrity of kit components is guaranteed for up to six months from date of purchase.
Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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