寻因生物
SEEKGENE

User Manual

SeekOne™ DD Training Kit

V1.1

REF: K01001-04 (4 tests)

Revised date: November 2023

Envision the Future

Beijing SeekGene BioSciences Co.,Ltd

Table of Contents

1. Introduction

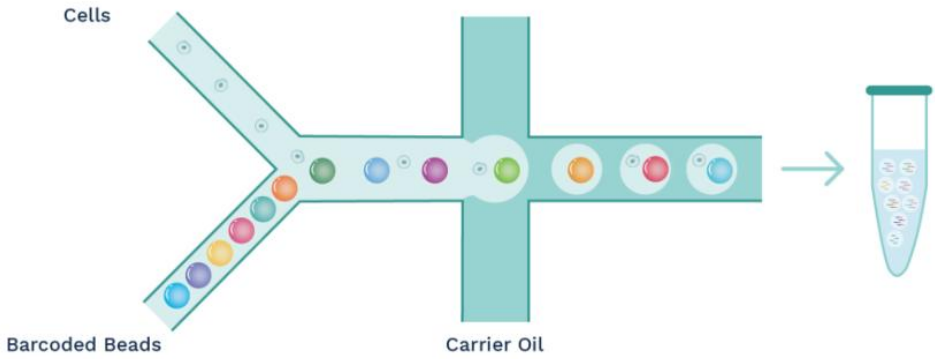
1.1 Overview

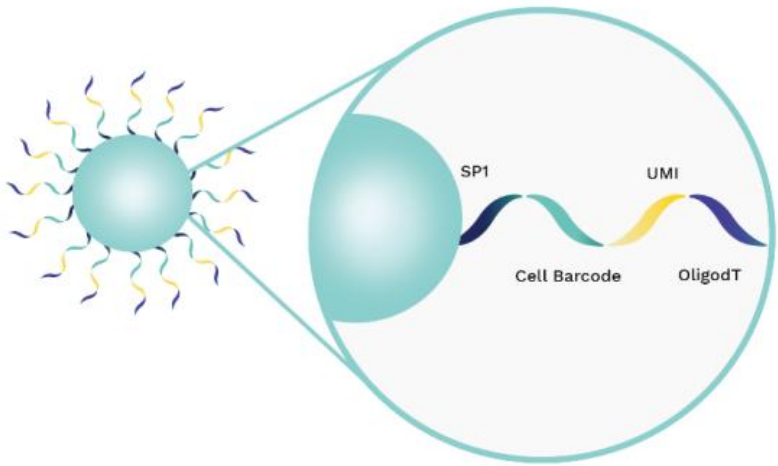
The SeekOne™ DD (Digital Droplet) Training Kit is designed by Beijing SeekGene BioSciences Co., Ltd.(abbreviated as SeekGene) to train new users in water-in-oil generation and barcode labeling experiments.This reagent kit is intended for use with the SeekOne™ Digital Droplet System (abbreviated as SeekOne™ DD, REF: M001A).

SeekOne™ DD Training Kit includes: chip (SeekOne™ DD Training Chip, referred to as Chip T), gasket, carrier oil, gel beads (SeekOne™ DD Training Beads, abbreviated as Training Beads).

The reagent kit is exclusively intended for training purposes, and **cannot substitute for formal reagents in experimental procedures.**

1.2 Experimental Principles



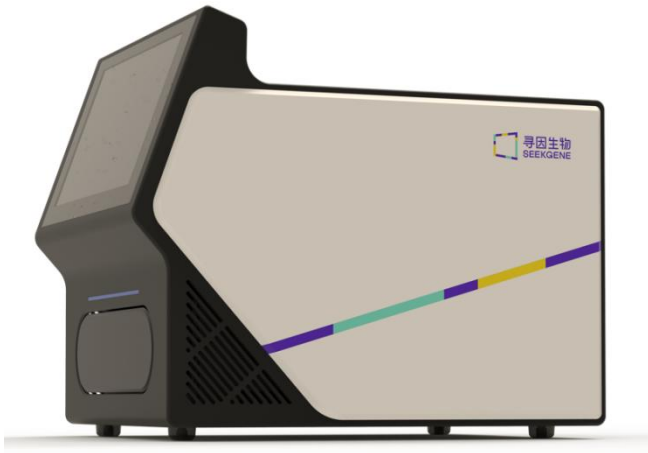


1.3 Product Components and Storage Conditions

Name & REF	Tube lid color	Components	CN	Volume	Storage Conditions
SeekOne™ DD Training Kit, **K01001-04, 4 tests**	-	SeekOne™ DD Training		4 pieces	Ambient
	-	Gasket		4 pieces	Ambient
	●	Carrier Oil		1.2 mL	Ambient
	○	SeekOne™ DD Training		200 μL	4˚C
	●	Training Sample Mix		400 μL	4˚C

1.4 Compatible Instruments and Consumables

1) SeekOne™ Digital Droplet System (SeekOne™ DD, REF: M001A)



2) SeekOne™ DD Accessories：Each instrument is equipped with one set of this accessory, which includes the following two parts:

a. SeekOne™ DD Chip Holder, Abbreviated as Chip Holder： used in conjunction with SeekOne™ Digital Droplet System and Chip S3.

b. Placed Chip, Abbreviated as Chip P：Placed in the chip fixture (8 Chip Ps are included with each instrument). When the sample size is less than 8, the Chip P is used and placed at the position where no sample is added. It serves as a replacement for Chip S3.

1.5 Additional Equipment & Consumables

Item	Models	Supplier
Single Channel Pipettes	0.1-2.5 μL, 2-20 μL, 20-200 μL, 100-1,000 μL	Various
Pipette Tips	2.5 μL, 20 μL, 200 μL, 1000 μL	Rainin / Axygen Or appropriate sterile, DNA low-binding, and filtered pipette tips.
0.2 mL PCR tubes	0.2 mL	Various, DNA low-binding tubes
Mini Centrifuge	-	TIANGEN, OSE-MP25,
Vortex Mixer	-	Various

2. Training Steps

Preparation Before Experiment

☐ Prepare a box with ice in advance.

☐ Take out the Training Sample Mix from 4℃ in advance, vortex thoroughly, centrifuge briefly, and place them on ice until use.

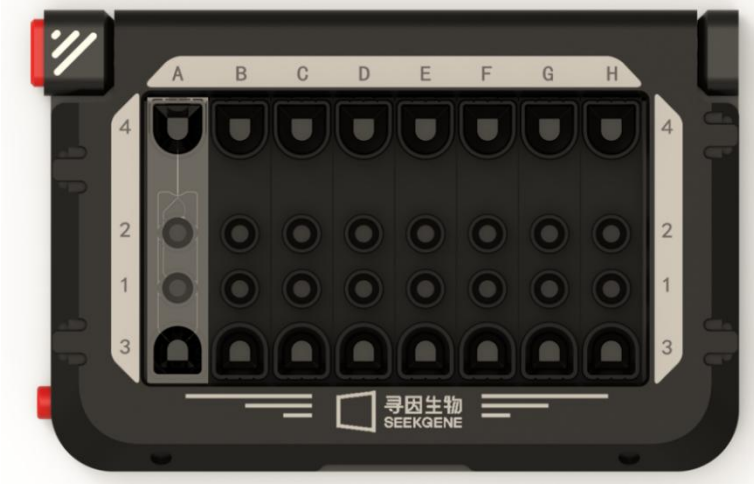
☐ Remove Training Beads from 4℃ in advance and equilibrate at room temperature for 30 minutes. Put back to 4℃ immediately after use.

☐ Ensure that the SeekOne™ Digital Droplet System is placed horizontally, operating at room temperature, and free from vibration or collision.

☐ Turn on the SeekOne™ Digital Droplet System, place the Placed Chip (Chip P), and run the self-check program. Wait for the self-check to succeed before proceeding with the experiment. **Do not shake or move the instrument while it is running.**

Step 1 Add Reagent to Chip T

1. Take out the necessary amount of Chip T from the package, one Chip T is needed for each sample (e.g. 4 samples, take out 4 Chip T). Place them into the slots of the Chip Holder by pressing the red button on the left to allow loading of the Chip T. For all remaining places, insert the Chip P (if there are only 4 samples, 4 positions are free, fill these with Chip P). Then close the cover of the Chip Holder (as shown in the figure below). Make sure that all positions contain a Chip.



Note 1**: If there are less than 8 samples, the empty chip positions must be replaced with Chip P. The 8 chip positions in the chip holder should not be left empty.* ***But nothing is pipetted into Chip P!

***Note 2**: Take out the amount of Chip T according to the number of samples from the plastic bags and use them within 24 hours after opening to avoid dust or other contamination.*

2. Pipette and mix the Training Sample Mix by pipetting it up and down 15 times with a pipette. Take 78 μL of the Training Sample Mix and insert the tip of the pipette tip into the Well 1, hold it slightly tilted into the bottom center of the well, slightly above the very bottom of the well, pipette slowly without bubbles, and let the mix stand for 30 seconds.

3. Vortex the Training Beads well at room temperature for 30 seconds, briefly centrifuge for 5 seconds, ensure that there are no air bubbles in the Barcoded Beads liquid, and pipette 40 μL into the Well 2. The tip should be inserted slightly tilted into the bottom center and slightly above the very bottom of the well, and pipette slowly without generating air bubbles.

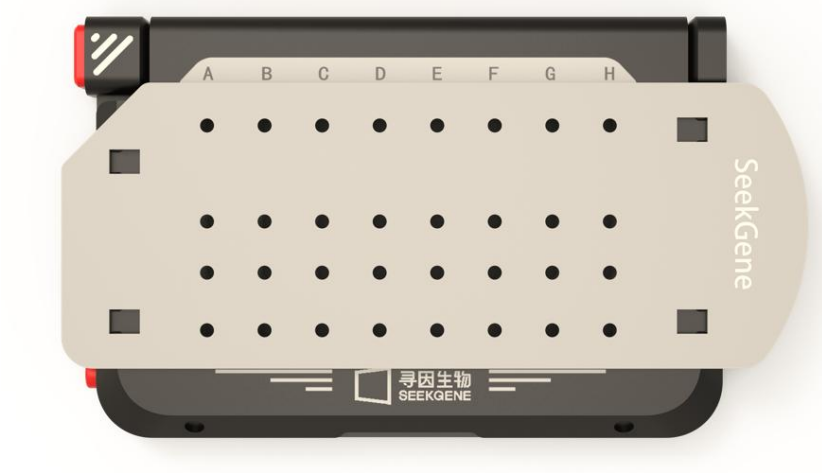
***Note 1:** When adding reagents, keep the pipette tip moving with the liquid level and always ensure that only the tip of the pipette is below the liquid surface by 3 mm to avoid generating bubbles.*

***Note 2:** The Training Beads liquid is viscous. After pipetting the designated volume, leave the pipette tip in the reagent tube for 5 seconds before removing it for sample addition.*

4. Pipette 120 μL of Carrier Oil with a 200 μL pipette into the Well 3, lean the tip against the inner wall, and pipette slowly without generating air bubbles. Repeat this step for a total of 240 μL of Carrier Oil in Well 3.

***Note**: Adding Carrier Oil improperly may fail water-in-oil droplet generation or damage the instrument.*

5. Attach the Gasket over the Chip Holder as shown in the illustration below, ensuring that the Gasket holes and the chip wells are aligned. The cut-off corner should be on the left upper side.



***Note**: Do not touch the smooth surface of Gasket.*

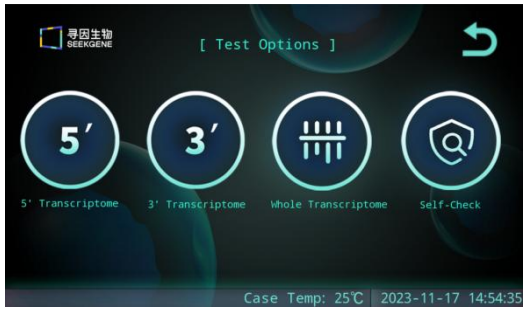
Step 2 Run SeekOne™ DD

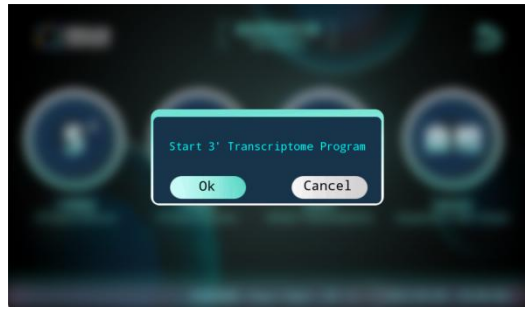
1. Click the "Open Chip Compartment" button on the SeekOne™ DD to eject the tray.

2. Put the Chip Holder with the covering Gasket into the tray according to the illustration, make sure the Chip Holder is placed horizontally, click the "Close Chip Compartment" button to retract the holder tray.

3. Click the "3’ Transcriptome" program and the "OK" button on the instrument screen to initiate the program.

Note: Select the appropriate program based on your experimental requirements. ***Do not select the “Self-Check” program.***





4. After the program is finished, click the "Run Completed" button and remove the Chip Holder. Immediately proceed to the next step.

Step 3 Transfer the Resulting Water-in-oil

1. Place a new 0.2 mL PCR tube on ice.

2. Click the "Open Chip Compartment" button on the SeekOne™ DD to eject the tray. Discard the Gasket, press and hold the square PUSH button, and open the Chip Holder cover all the way, until the cover is at a 45° horizontal angle shown in the picture:

3. Wells 1 and 2 had the Training Sample Mix and Training Beads. Abnormally high volume in either well indicates a clog.

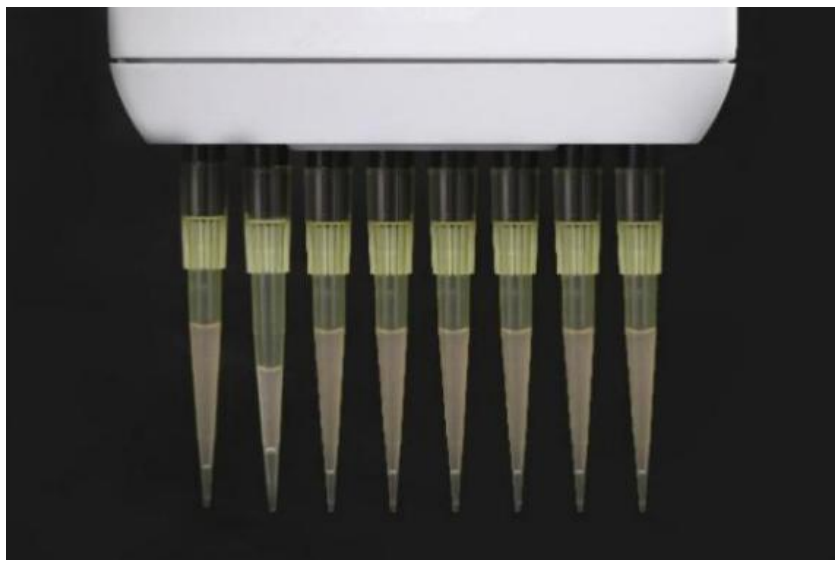
***Note**: The remaining volume of well 1 (aqueous phase) should not be more than 10 µL. If the remaining volume of well 2 (adhesive bead phase) is greater than 15 µL, this indicates that the chip is blocked.*

4. Use a pipette to **slowly** aspirate all (at least 120 µL) the water-in-oil liquid from the Well 4.

***Note 1:** When pipetting out water-in-oil emulsion, the tip of the pipette tip should be kept suspended in the liquid without touching the bottom of the well. If there is any excess carrier oil (clear) at the bottom, it can be removed using a 0.5-10 µL pipette tip, taking care not to aspirate the pink water-in-oil liquid.*

***Note 2**: When running multiple chips simultaneously, there is a low probability of observing bubbles in the Wells 4 in individual chips. This is a normal occurrence and does not affect the library preparation and subsequent results.*

5. Observe the liquid inside the pipette tip, normal liquid phase (upper phase) should appear uniformly opaque and turbid. Excess Carrier Oil (clear) in the pipette tips indicates a potential clog.



***Note**: If the solution is like in the second tip from the left in the figure, it indicates a clog.*

6. Slowly (~20 sec) pipette the water-in-oil from the pipette tip into the 0.2 mL PCR tube placed on ice, by pipetting it along the wall of the tube.

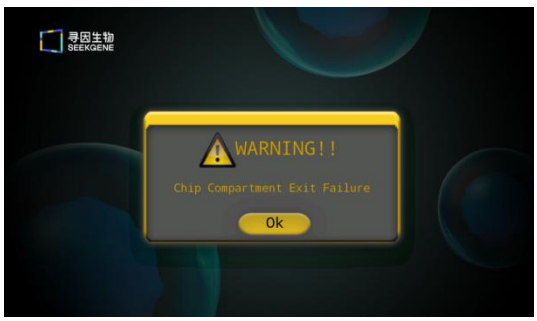
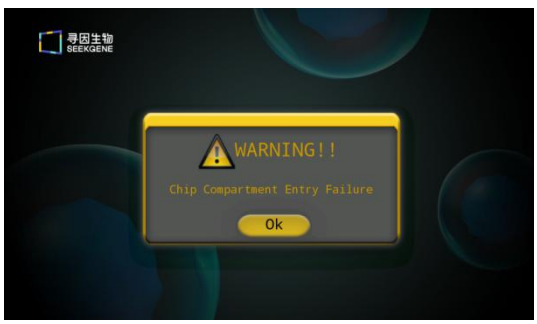
***Note**: This concludes the Training Kit protocol. This training protocol does not proceed with cDNA amplification or any other steps outlined in other User Manuals.*

Appendix 1: SeekOne™ Digital Droplet System User Manual

Refer to *SeekOne™ Digital Droplet System User Manual*

Appendix 2: SeekOne™ Digital Droplet System Troubleshooting

Problems may occur during the operation of the equipment. The following table describes the fault types and how to deal with them. When the equipment malfunctions occur, the user can first troubleshoot and deal with it according to the following table, if the issue cannot be solved, please contact our company in time.

Failure type	Solution
	Please make sure the device is installed correctly, Click “OK” to perform a self-check, or restart the device. If this message appears repeatedly, it may indicate an internal hardware issue. Continued use under these circumstances can result in damage to the instrument. Please contact (info@seekgene.com) for further assistance.
	The operation in and out of the warehouse may be blocked. Please confirm that no objects are blocking the running path and click the "OK" button on the prompt window. The instrument will proceed to the next step. If the message appears repeatedly, please contact (info@seekgene.com) for further assistance.
	The operation in and out of the warehouse may be blocked. Please confirm that no objects are blocking the running path and click the "OK" button on the prompt window. The instrument will proceed to the next step. If the message appears repeatedly, please contact (info@seekgene.com) for further assistance.
	Please try again or restart the operation. If the message appears repeatedly, please contact (info@seekgene.com) for further assistance.

	Please verify if the gasket is properly seated on the Chip Holder and reposition the Chip Holder. Check if there are any foreign objects on the surface of the chip compartment and clean the surface. If the message appears repeatedly, please contact (info@seekgene.com) for further assistance.
	Restart the device, if it recurs, contact (info@seekgene.com) for further assistance.
	Please check if the sealing gasket is clean, if the chip has any damage on its surface, and if the Chip Holder is installed correctly. If there is dirt in the sealing gasket or damage on the chip’ s surface, please replace the gasket or chip and try again. If the error message recurs, contact (info@seekgene.com) for further assistance.
	Please check if the sealing gasket is clean, if the chip has any damage on its surface, and if the Chip Holder is installed correctly. If there is dirt in the sealing gasket or damage on the chip’s surface, please replace the gasket or chip and try again. If the error message recurs, contact (info@seekgene.com) for further assistance.