ZellChip Technologies Inc.

Single Cell Biochip Kit-SCK

Catalog number: 15-12-1001

Intended Use

ZellChip Technologies Inc. Single Cell Biochip Kit is intended for **in vitro diagnostic use** as a tool for cell observation and measurement of drug uptake in a single cell. The kit is optimized for use with a fluorescent microscope.

Summary and Explanation

ZellChip Technologies Inc. Single Cell Biochip kit is suitable for isolating most human or animal cells, observing individual cells and measuring the drug uptake in them. (The intensity of fluorescence dye in the cell is related to the drug uptake). Interpretation of the results can only be made by qualified and trained personnel.

Principle of the Procedure

The Single Cell Biochip allows for isolation of the live cell while used in the procedure. This allows for interaction of reagents with the cell and measurement of cellular drug uptake depending on the primary use of the Single Cell Biochip.

Materials and Methods

Reagents

The kit contains 2 reagents in powder and 1 reagent in solution:

 Reagent A : 1 mg Reactive Component: Fluorescent Dye Formula weight: 380.82 g/mol Appearance: Dark red–brown powder Solvent: DMSO or EtOH

2. Reagent B: 10 mg

Reactive Component: Uptake Enhancer Formula weight: 733.93 g/mol Appearance: White or faint yellow powder Solvent: Deionized water

3. Solution C 10x : 1 mL

Reactive Component: Buffer 10X pH: 7.2-7.6 Appearance: Clear, Colorless Add 9 mL deionized water and mix

MSDS is available from ZellChip Technologies Inc. upon request.

Kit Capacity: Each set of biochips and reagents is capable of conducting 20 assays.

Warnings and Precautions

- Observe all federal, provincial, and local regulations when storing and disposing of substances. If this information is not available locally, contact ZellChip Technologies Inc. for recommendations.
- Contents of this kit must be handled and used by following good laboratory practices (e.g., gloves, lab coat, goggles, fume hood or filtered enclosure).
- Do not inhale powder. Avoid contact with skin, eyes, and clothing.
- Ensure that an eyewash station is available and maintained.
- For more information about first aid, fire fighting, and accidental release measures, refer to MSDS for kit reagents

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Caution: Fluorescence dye is harmful if swallowed. There is a risk of damage to eyes. For further information, see MSDS.

Storage Information

Store at room temperature. Keep containers tightly closed and protect them from physical damage before use. The contents must be used by the expiry date shown on the box label.

Upon Receiving:

- Open the package.
- Check that the content is intact.
- If the integrity of the vials has been compromised, or the Single Cell Biochip is broken, contact ZellChip Technologies Inc.

Specimen Collection and Preparation for Analysis

A variety of human or animal cells (not provided) can be used to perform assays.

Procedure

For professional use only.

The procedures described in this manual must be performed by qualified laboratory personnel. Directions outlined must be followed closely and precisely.

If the assay is interrupted, materials become contaminated or if it is not possible to continue procedure in a timely fashion, dispose of the spoiled batch you are working on.

Other Required Materials (not provided in the kit)

- Dimethyl sulfoxide (DMSO) or Ethanol (EtOH)
- Deionized water
- cell samples (density of 50,000-100,000 per mL)
- Pipettor and pipette tips (10, 200, 1000 uL)
- 0.6-mL and 1.5-mL vials

1. Solution C : Buffer 1X

- a. Add 9 mL of deionized water to buffer C 10X
- b. Shake well

2. Reagent A : Fluorescent dye

- a. Add 500 uL of DMSO or Ethanol to make a stock of 2 mg/ml
- b. Aliquot into 20 vials of 25 uL each using 1.5-mL vials **and store them at -20** °C until ready to use.
- c. When use, add 975 uL buffer C 1X into the vial to make a solution of 50 ug/mL
- d. This solution will be further diluted 25 times to 2 ug/mL by buffer C 1X.
 For instance, mix 20 uL of the 50-ug/mL solution with 480 uL buffer C 1X.

3. Reagent B : Uptake Enhancer

- a. Add 500 uL of DMSO or Ethanol to make a stock of 20 mg/mL.
- b. Aliquot into 20 vials of 25 uL each using 1.5-mL vials and store them at -20 °C until ready to use.
- c. When use, add 475 uL buffer C 1X into the vial to make a solution of 1 $\,mg/mL$
- d. This solution will be further diluted 5 times to 200 ug/mL by buffer C 1X . For instance, mix 20 uL of the 1-mg/mL solution with 80 uL buffer C 1X.

Procedure: Measurement of drug uptake in a cancer cell

- 1. Take a Single Cell Biochip out of the package
- 2. Put the Biochip on the bench so that the Chip Label faces up and is on the left side, see Fig 1. below
- 3. Put in 2 uL of cell suspension (density of 50,000-100,000 per mL) into reservoir 1
- 4. Put in Solution Buffer C 1X (2 uL) in reservoir 3
- 5. Observe the flow of cells into the cell retention structure S toward reservoir 2, but not reservoirs 3 and 4.
- 6. Adjust the amount of liquid (in portions of 0.5 uL) put in reservoir 1 to increase or in reservoir 2 to decrease the liquid flow
- 7. When a desired cell reaches the entrance of the retention structure S, add Solution Buffer C 1X (1 uL) in reservoir 3 to push the cell into the retention structure
- Add a solution of reagent A (3 uL) to reservoir 4. Note that 3 uL reagent A (2 ug/mL) in reservoir 4 and 3 uL buffer C 1X in reservoir 3 will result in 1 ug/mL reagent A interacting with the cell.
- 9. Note the fluorescent intensity of the cell, image after 15 min.
- 10. Replace reservoir 3 with 3 uL of reagent B solution. Note that 3 uL reagent A (2 ug/mL) in reservoir 4 and 3 uL reagent B (200 ug/mL) in reservoir 3 will result in 1 ug/mL reagent A plus 100 ug/mL reagent B interacting with the cell.
- 11. Step 10 can be replaced with the uptake enhancer solution chosen by the user.
- 12. Note the fluorescence intensity of the cell for a change, image after 15 min.

4 3 4 3 1 5 2



Fig 1. Single Cell Biochip

Quality Control Procedures

Any fresh cell suspension of human or animal origin (not provided) can be used with the Single Cell Biochip Kit. Each cell type may require that an individual laboratory protocol is implemented. If necessary you may contact ZellChip Technologies Inc.

Expected Results:

For a cell with good drug uptake, bright fluorescence due to reagent A is observed

Limitations of the Procedure

- The procedures described in this manual must be performed by qualified and trained laboratory personnel.
- Optimal assay results are dependent on cell quality; improper handling or processing may introduce result artifacts.
- Interpretation of the results must be made by authorized and trained personnel and only when appropriate controls are used.

Symbols used on the kit labels

REF	Manufacturer's Catalog Number
LOT	Batch Code
><	Use By (Expiry Date)
***	Manufacturer
ND	In Vitro Diagnostic Medical Device
i	Consult Instructions For Use

Contacting ZellChip Technologies Inc.

For a troubleshooting guide and list of recommended supplies, contact ZellChip Technologies Inc. or visit www.zellchip.com

ZellChip Technologies Inc. 8900 Nelson Way, Burnaby BC Canada V5A 4W9 Tel. 778-782-9451 Website: www.zellchip.com

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