DATA SHEET

Section 1. Product and Company Identification

Product Name: QCell-Eva One-Step qRT-PCR Kit
Catalog Number: K5054200, K5054400

MSDS# K5054200, K5054400
Component/Item (and Parts number if listed)

Components	Part No.
1. Cell Lysis Buffer	K5054200-1, K5054400-1
2. Eva qRT-PCR Reaction Mixture, 5x (containing Eva Dye and Hotstart Taq DNA polymerase)	K5054200-2, K5054400-2
3. Reverse Transcriptase / RNase Inhibitor Mixture	K5054200-3, K5054400-3
4. ROX Reference Dye	K5054200-4, K5054400-4
5. Human GAPDH control F/R primer pair (25x)	K5054200-5, K5054400-5
6. Nuclease-Free PCR Grade Water	K5054200-6, K5054400-6

Shipping Condition: Dry ice

Storage Condition: –20 °C protected from light

Shelf Life: One year from the date of receipt under proper storage conditions

Description

Components in this kit are prepared with pure chemicals according to our proprietary technology. QCell-Eva One-Step qRT-PCR SuperMix Kit provides a one-step, simple, robust, inexpensive assay for detection and quantitative analysis of gene expression directly from cells or RNA with intercalator format.

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1910 Kampenhout, Belgium

Section 2. Composition and Information on Hazardous (OSHA) Ingredients

Components 1 – 5: Irritating to eyes, respiratory system and skin.

Section 3. Hazards Identification

Review approved and the most current institutional guideline, protocol, SOP(s)and MSDS(s)for the proper handling of institutional materials/equipment associated with the use of this BCI product.
Routes of Entry: *Skin Absorption* ***(No);*** *Dermal/skin contact* ***(Yes);*** *Eye contact* ***(Yes);*** *Inhalation* ***(No);*** *Ingestion* ***(Yes);*** *Chronic Exposure* ***(No).***
Potential Acute Health Effects: Avoid contact with eyes, skin and clothing. Wash thoroughly after handling. Slightly hazardous in case of skin contact (irritant). Hazardous in case of eye contact (irritant). Slightly hazardous in case of ingestion.
Carcinogenic Effects: Not listed by NTP, IARC or OSHA.
Mutagenic Effects: Not available.
Teratogenic Effects: Not available.

Section 4. First Aid Measures

Skin: Wash exposed skin with mild soap and water. Get medical attention if irritation develops or persists.
Eyes: Immediately flush with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Seek medical attention.
Ingestion: If affected person is conscious, give plenty of water to drink. Seek medical attention. Wash contaminated clothing before reuse.

Section 5. Fire Fighting Measures

Flammability of the Product: Not flammable.

Flash Points: Not available.
Fire Fighting Procedures:
Small Fire: Use DRY chemical powder.
Large Fire: Use water spray, fog or foam. Do not use water hose.

Section 6. Accidental Release Measures

Small Spill and Leak:
Absorb with an inert dry material and place in an appropriate waste disposal container. Finish cleaning by spreading water on the contaminated surface and dispose of according to local and regional authority requirements.

Section 7. Handling and Storage

Handling: Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling.
Storage: store at -20°C and protected from light.

Section 8. Exposure Controls/Personal Protection

Ensure that eyewash stations and safety showers are proximal to the work-station location.
Eyes and Face: Splash goggles.
Body: Lab coat.
Respiratory: Be sure to use NIOSH/MSHA approved respirator or equivalent. Wear appropriate respirator when ventilation is inadequate.
Hands: Gloves.
Consult local authorities for acceptable exposure limits.

Section 9. Physical and Chemical Properties

Components/Item	Physical state	Color	odor
1. Cell Lysis Buffer	Liquid	Clear	None
2. Eva qRT-PCR Reaction Mixture, 5x (containing Eva Dye and Hotstart Taq DNA polymerase)	Liquid	Clear	None
3. Reverse Transcriptase / RNase Inhibitor Mixture	Liquid	Clear	None
4. ROX Reference Dye (50x)	Liquid	Red	None
5. Human GAPDH control F/R primer pair (25x)	Liquid	Clear	None
6. Nuclease-Free PCR Grade Water	Liquid	Clear	None

Section 10. Stability and Reactivity

Stability and Reactivity: The product is stable
Incompatibility with Various Substances No decomposition if used according to specifications. No dangerous reactions known.

Section 11. Toxicological Information

Primary Irritant Effects: Harmful if ingested.
On the Eye: Cause eye irritation.
Sensitization: No sensitizing effects known.

Section 12. Ecological Information

Data not yet available.

Section 13. Disposal Considerations

Waste Information:
Must not be disposed of together with household garbage. Do not allow product to reach sewage system.
Hand over to hazardous waste disposers.
Waste must be disposed of in accordance with federal, state and local environmental control regulations.

EINECS: Not available.
European Information: Irritating to eyes, skin, and respiratory system. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Validated by BCI Administration on 10/06/06 . **Verified by BCI Administration.**
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Notice to Reader
The information contained in this MSDS was obtained from sources we believe are reliable. However, the above information is provided without warranty, expressed or implied, regarding its correctness. The conditions or methods if handling, storage, use and disposal of the product are beyond our control and may be beyond our knowledge. Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

User's Manual and Instructions

Product: QCell-Eva One-Step qRT-PCR SuperMix Kit

Catalog Number: K5054200, K5054400

Introduction
qRT-PCR is a highly sensitive technique that is widely used for detection and quantification of RNA in tissues and cultured cells. Traditionally, quantitative PCR is performed in two steps: a first-strand cDNA synthesis step using reverse transcriptase, followed by a PCR step using a thermostable DNA polymerase. This Kit combines Reverse Transcriptase (MMLV-RTase) and RNase Inhibitor in a single mixture, with Eva fluorescent dye and hotstart Taq DNA polymerase in a separate 2x reaction mix optimized for qRT-PCR. Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and either cell lysate or RNA. A cell lysis buffer is provided in the kit to make cell lysates in less than 5 minutes at room temperature. The cell lysate can be used directly for qRT-PCR, bypassing RNA isolation procedure. The passive reference dye ROX is included in a separate tube to make the QCell-Eva One-Step qRT-PCR SuperMix adaptable for many real-time QPCR platforms. Human GAPDH primer set for RT-PCR is also included in the kit as a control. This primer was designed to span an exon-exon boundary in the human GAPDH cDNA, which can eliminate the undesired amplification of genomic DNA in the RNA or cell lysate.

BioChain's QRT-PCR SuperMix contains BioChain's Taq polymerase with hot start capability. BioChain's hot-start Taq polymerase improves PCR amplification reactions by decreasing non-specific amplification and preventing primer-dimer formation. This enzyme is activated after an initial 10 minutes heating at 95°C. And the real-time RT-PCR buffer is specially formulated to provide superior specificity and increase reverse transcription and amplification efficiency.

Eva Dye

Eva Dye binds double–stranded DNA. Detection is monitored by measuring the increase in fluorescence intensity throughout the cycle. Eva Dye has higher affinity to double-stranded DNA than SYBR Green dye and shows stronger fluorescence intensity than SYBR Green upon binding to DNA. Eva Dye is more stable than SYBR Green and the absorption and emission spectra of Eva Dye are very similar to SYBR Green Dye or FAM, so the same optical setting for SYBR Green Dye or FAM can also be used for Eva Dye.

Features

- Flexible and convenient – quantitating gene expression in cells (without isolating RNA) or RNA in one-step format
- Save time – quick cell lysis procedure, and ready-to-use supermix reducing setup time and liquid handling steps
- High Sensitivity – qRT-PCR from as low as 1 cell or 1 pg total RNA.
- Versatile – compatible with a wide variety of cell lines

Applications

- Real-Time RT-PCR
- Gene expression profiling
- Gene knockdown verification
- Array Validation

Quality Control

1 kit of this lot has been tested for quantitating human GAPDH gene expression in a serial dilution of cell lysate from 64 cells to 1 cell using Stratagene's Mx3005P as a real time PCR instrument. Good linearity and great PCR efficiency is observed and consistent with the previous lot.

Components

Catalog Number: K5054200: Reagents are sufficient for 200 assays

Item	Amount	Part No.
1. Cell Lysis Buffer	20 ml	K5054200-1
2. Eva qRT-PCR Reaction Mixture, 2x (containing Eva Dye and Hotstart Taq DNA polymerase)	1.25 ml x 2	K5054200-2
3. Reverse Transcriptase / RNase Inhibitor Mixture	100 µl	K5054200-3
4. ROX Reference Dye	50 µl x 2	K5054200-4
5. Human GAPDH control F/R primer pair (25x)	200 µl	K5054200-5
6. Nuclease-Free PCR Grade Water	3 ml	K5054200-6

Catalog Number: K5054400: Reagents are sufficient for 400 assays

Item	Amount	Part No.
1. Cell Lysis Buffer	40 ml	K5054400-1
2. Eva qRT-PCR Reaction Mixture, 2x (containing Eva Dye and Hotstart Taq DNA Polymerase)	1.25 ml x 4	K5054400-2
3. Reverse Transcriptase / RNase Inhibitor Mixture	100 µl x 2	K5054400-3
4. ROX Reference Dye	50 µl x 4	K5054400-4
5. Human GAPDH control F/R primer pair (25x)	200 µl	K5054400-5
6. Nuclease-Free PCR Grade Water	3 ml x 2	K5054400-6

Reagents and Equipments Required but not Supplied in this Kit:

1. PBS (Ca^{2+}, Mg^{2+} free)
2. Spectrofluorometric thermal cycler

Storage and Stability

Upon receipt, store all components at -20 °C in a constant temperature freezer. Avoid repeated freeze/thaw cycles. When stored under these conditions the supermix is stable for one year after ship date. The Eva Dye and the ROX reference dye are light sensitive and should be kept away from light whenever possible.

Protocol

1. Harvest cells using the method appropriate to the properties of the cell line. For adherent cells, trypsinize the cells using standard techniques. Count the cell.
2. Pelleting the cells by centrifuging at 200 – 300x g for 5 min. Carefully remove the supernatant by aspiration.
3. Wash the pellet once with ice-cold PBS. Pelleting the cells by centrifuging at 200 – 300x g for 5 min. Carefully remove the supernatant by aspiration. Keep the pellet on ice.
4. Add appropriate volume of Cell Lysis Buffer to the cell pellet. Vortexing for 1 minute to lyse the cells.
5. Analyze the lysate by qRT-PCR. RNAs in the lysate are stable at 4°C for up to 4 hr.

QRT-PCR setup and cycling

1. Prepare the following RT-PCR reaction mixture. (First make the master mix without the template. After making the master mix, gently mix the reaction without creating bubbles, aliquot and then add 1 – 2.5 µl of template to each experimental reaction)

per reaction: 25 µl

Regents	Volume	Final Concentration
Eva QRT-PCR Reaction Mixture (2x)	12.5 µl	1x
Reverse Transcriptase / RNase Inhibitor Mixture	0.5 µl	
PCR forward primer	X µl	150 – 200 nM
PCR reverse primer	X µl	150 – 200 nM
ROX Reference Dye [a]	0.5 µl	
Template (cell lysate or RNA)[b]	1 – 2.5 µl	
Nuclease-free PCR grade water	Add up to 25 µl	

[a] See page 5: Use of the ROX Reference Dye

[b] If cell lysate is used as the template, the volume of cell lysate should not exceed 1/10 volume of the qRT-PCR reaction. If RNA is used as the template, it is recommended to use RNA template in less than 250 ng.

2. Gently mix the reactions without creating bubbles since bubbles interfere with fluorescence detection. Then centrifuge the reactions briefly.

3. Place the reactions in the instrument and run the appropriate RT-PCR program. Try the following protocol first, and optimize the reaction conditions if needed.
PCR program for RT-PCR:

Cycles	Temperature	Time	Detection	Remark
1	42°C	15 min	OFF	
1	95°C	10 min.	OFF	This step inactivates the reverse transcriptase and activates the hotstart Taq DNA polymerase. 10 minutes incubation is required to fully activate hotstart Taq DNA polymerase.
40	95°C	15 sec	OFF	
	50-60°C [a]	15 sec	ON	
	72°C	30 sec	OFF	

a. Set an appropriate annealing temperature for the primer set used.

4. Dissociation Program for all PCR products

Follow manufacturer's guidelines for setting up dissociation depending on the instrument's software version.

QRT-PCR Setup and Cycling Program for human GAPDH control primer set (amplicon size = 226 bp)

1. Prepare the following RT-PCR reaction mixture. (First make the master mix without the template. After making the master mix, gently mix the reaction without creating bubbles, aliquot and then add 1 – 2.5 µl of template to each experimental reaction).

per reaction: 25 µl

Regents	Volume
Eva QRT-PCR Reaction Mixture (2x)	12.5 µl
Reverse Transcriptase / RNase Inhibitor Mixture	0.5 µl
Human GAPDH primer set (25x)	1 µl
Reference Dye ROX [a]	0.5 µl
Template	2.5 µl
Nuclease-free PCR grade water	Add up to 25 µl

[a] See page 5: Use of the ROX Reference Dye

2. PCR program for amplification of human GAPDH amplicon.

Cycles	Temperature	Time	Detection
1	42°C	15 min	OFF
1	95°C	10 min.	OFF
40	95°C	30 sec	OFF
	55°C	15 sec	ON
	72C	30 sec	OFF

3. Dissociation Program: Follow manufacturer's guidelines for setting up dissociation depending on the instrument's software version.