SensiDeath

Protocol

Protocol overview

Lyse your sample

1

Using our special lysis buffer disrupting cell plasma membrane without altering that of nucleus and organelles

20 min

Collect cytosolic fractions

2

Using a simple centrifugation step

5 min

Cytosolic fractions can be stored at < -20°C and processed later if needed

Amplify

3

Specific genomic sequences by quantitative PCR using our optimized primers

2 h

Assess cell death

By quantifying cytosolic enrichment in genomic DNA

1. Description

During cell death, fragmented genomic DNA accumulates in the cytosol. Using a selective lysis buffer and qPCR primers, SensiDeath allows a rapid and highly sensitive cell death quantification of any human cultured cells. Conversely, it can also be used to determine treatments or culture conditions promoting cell survival.

2. Kit contents

Provided in the kit:

- 6X Lysis Buffer
- 20X Primer Mix

To be provided by the user:

- PCR-grade water
- SYBR Green qPCR Master mix*
- DNA-free tubes (1 per sample)

*any SYBR Green dye-based qPCR mix can be used with the provided protocol.

3. Storage

- Store the 6X Lysis buffer at 4°C for up to 1 year.*
- Store the 20X primer mix at 4°C for up to 1 month, or aliquot and keep at -20°C for long-term storage.

*some precipitates may form in the Lysis Buffer upon storage. If needed, heat at 95°C before use until fully dissolved.

4. Cell lysis

All following steps should be performed at room temperature.

- Seed your cells in culture plates and treat them according to your specific experimental conditions.

If working with suspension cells, aim for a final concentration of approx. 0.1 x 10^6 cells/mL.

Remember to include an untreated control, which will serve as a baseline to determine cell death levels during qPCR analysis.

The lysis buffer can either be added to the cultured cells alone or directly to the full cell culture medium.

Option 1: Adding the lysis buffer to cultured cells

- Prepare your required volume of 1X Lysis Buffer working solution by diluting the 6X Lysis Buffer provided in PCR-grade water to make a 6-fold dilution.

- Remove all culture medium from the cells.

If working with suspension cells, centrifuge the plate or vial for 5 min at 200g to pellet cells before removing the culture medium.

- Directly add 1X Lysis buffer to the cells to get a final lysate concentration of 50-200 cells/µL (i.e. 50µL/well for 96-well plates, or 100µL/well for 48-well plates).

It is not necessary washing the cells before adding the lysis buffer. If a washing step is needed, we recommend using HEPES buffer instead of PBS.

- Incubate for 15 min at room temperature.

Option 2: Adding the lysis buffer to the full culture medium

- Directly add your 6X Lysis Buffer to your culture medium at a 1:6 ratio to achieve a final 1X concentration.

- Incubate for 15 min at room temperature.

5. cytosolic fraction recovery

- Transfer each lysate to a DNA-free tube and centrifuge at 2,000g for 5 min.

- Collect up to 50% of the supernatant and transfer it to a fresh DNA-free tube.

We recommend not taking more than 50% from the upper half to make sure no cellular debris or nuclear contents are collected. This volume should be more than enough to perform subsequent qPCR assays.

At this stage, cytosolic fractions can be stored at < -20°C and processed later.

6. qPCR & data analysis

- Prepare your qPCR reactions by mixing your qPCR Master Mix with the cytosolic fractions previously obtained and the 20X Primer Mix provided in the kit (cf Table 1).

Table 1. Volumes to use for each component in final qPCR reactions

Component	Volume (per 20 µL qPCR reaction)
qPCR Master Mix	According to manufacturer's protocol
20X primer mix	1 µL
cytosolic fraction (diluted in PCR-grade water)*	qsp 20 µL
Total volume	20 µL

***Diluting cytosolic fractions in PCR-grade water**

Loading too much cellular material may cause system saturation and result in higher result variability. For optimal performance, we recommend using the equivalent of 5-10 cells per qPCR reaction.

If the number of cells is unknown, the optimal dilution ratio can be empirically determined by performing a dilution series between 1:25 and 1:100.

Similar ratios between treated *vs.* non-treated samples should be observed accross all dilution points (if a 1:25 dilution generates the same results as a 1:50 or 1:100 dilution, a 25-fold dilution is sufficient and can be selected for future assays).

- Set up and run the real-time PCR instrument as per the manufacturer's instructions, using a primer annealing temperature of 60°C and an extension temperature of 72°C.

- Cell death levels can be compared across samples based on the commonly used $2^{-\Delta Ct}$ method. The example below can be used as a reference to analyze your results.

It is advised to include a negative control sample, containing diluted lysis buffer alone instead of cytosolic fractions, in order to check for potential contaminations.

Example of cell death quantifications based on Ct values

Ct (untreated cells)	Ct (treated cells)
20.51	16.40
20.77	16.60

- Average Ct values from each duplicate

Mean Ct (untreated cells) = 20.64
Mean Ct (treated cells) = 16.5

- Apply a log-linear transformation of each Ct value (2^{-Ct})

Untreated cells = $2^{(-20.64)}$
Treated cells = $2^{(-16.5)}$

- Calculate the relative fold increase by normalizing your treated sample 2^{-Ct} values to the untreated control 2^{-Ct} value. This fold increase will be representative of the relative cell death variations across samples.

Untreated cells = $2^{(-20.64)}/2^{(-20.64)} = 1$

Treated cells = $2^{(-16.5)}/2^{(-20.64)} = 17.6$

The dynamic range is large and usually varies between 3 and 50-fold increase depending on the cell type used and the treatment applied.

7. Troubleshooting section

Issue	Comments & suggestions
I am getting some amplification signal in my no template controls	While it is common to expect a Ct >35 in no-template control samples with standard qPCR protocols, it is not unusual to get a higher Ct with the SensiDeath method. This is due to the fact that the SensiDeath primers were designed to amplify gDNA, and are much more sensitive than standard primers amplifying cDNA. Any slightest trace of contaminant gDNA present in the PCR mix, that might have not been picked up by other qPCR primers, will result in an amplification signal. To evaluate the accuracy of your results, the difference between the Ct value of your NTC *vs* that of your cytosolic samples (ΔCt) should be higher than 5 meaning that your non-specific signal accounts for <3% of your specific signal.
I am getting a large dissociation curve peak	The SensiDeath primers are designed to amplify a genomic repeated element which, although well-conserved throughout evolution, translates in 92% amplicons having the same size (153bp). This means that the dissociation curve peak obtained after amplification is expected to be slightly larger compared to primers amplifying a unique sequence. You should expect a single peak at around 81.5°C. The primers are not known to produce any dimers.

Issue	Comments & suggestions
I am not getting the expected correlation in my treatment dose-response curves / I am unsure about the accuracy of my results	The high sensitivity and early time window of the SensiDeath method are two important criteria to take into account when setting up a cytotoxicity assay. While other standard cytotoxicity assays are often based on the detection of large DNA fragments released out of the cell, SensiDeath will detect small genomic fragments released inside the cell cytosol as soon as the cell death process is initiated. If cytotoxicity is too high, or the cell death process too advanced, cells will likely be already lysed and their fragmented DNA released from the cytosol to the external medium. This can result in a lack of signal. If detecting late-stage cell death, adding the lysis buffer directly to the culture medium will allow you to make sure you detect all fragmented DNA that might have been released from the cells. Otherwise, reducing the treatment duration and/or concentration to catch up cell death earlier can also help improve your result consistency.

The SensiDeath technology was developed by Eric Lacazette, Henrik Laurell & Christian Touriol (Equipe ANUBIS, Université de Toulouse, France).

SENSIDEATH ONLINE

Find out detailed technical information, FAQ, result examples and much more at:

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