For life science research only.
Not for use in diagnostic procedures.

Cellular DNA Fragmentation ELISA

Version: 12
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Photometric enzyme-linked immunosorbent assay (ELISA) for the detection of BrdU-labeled DNA fragments in cell lysates or in cell culture supernatants.
A nonradioactive alternative to the [^{3}H]-thymidine release assay, the [^{3}H]-thymidine-based DNA fragmentation assay, and the [^{51}Cr]-release assay.

Cat. No. 11 585 045 001 1 kit
500 tests

Store the kit at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	white	Cellular DNA Fragmentation ELISA, Anti-DNA	▪ Lyophilized, stabilized ▪ Monoclonal antibody from mouse (clone MCA-33).	1 bottle
2	red	Cellular DNA Fragmentation ELISA, Anti-BrdU-POD	▪ Lyophilized, stabilized ▪ Monoclonal antibody from mouse (clone BMG 6H8, Fab fragment), conjugated with peroxidase.	1 bottle
3	white	Cellular DNA Fragmentation ELISA, Coating buffer, 10x conc.	For preparation of solution 3 and coating of the microplates.	1 bottle, 6 ml
4	green	Cellular DNA Fragmentation ELISA, Washing buffer, 10x conc.	Contains EDTA, Tween 20, and 0.01% 2-methylisothiazolone (MIT) as a preservative.	2 bottles, 100 ml each
5	red	Cellular DNA Fragmentation ELISA, Incubation buffer, 2x conc.	Contains BSA, EDTA, Tween 20, and 0.01% 2-methylisothiazolone (MIT) as a preservative.	1 bottle, 125 ml
6	white	Cellular DNA Fragmentation ELISA, Substrate solution	▪ Ready-to-use TMB solution. ▪ For ELISA and photometric measurement.	1 bottle, 55 ml
7	red	Cellular DNA Fragmentation ELISA, BrdU labeling reagent, 1,000x conc.	Contains 10 mM 5′-bromo-2′-deoxyuridine in PBS, pH 7.4, sterile.	1 bottle, 1 ml
8	–	Cellular DNA Fragmentation ELISA, Self-adhesive Plate Cover Foil	Prevents evaporation. ⚠ ***Cover the plates with the Cover Foils during each incubation step.***	10 foils

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	white	Anti-DNA	Store at +2 to +8°C.
2	red	Anti-BrdU-POD	
3	white	Coating buffer, 10x conc.	
4	green	Washing buffer, 10x conc.	
5	red	Incubation buffer, 2x conc.	
6	white	Substrate solution	
7	red	BrdU labeling reagent, 1,000x conc.	
8	–	Self-adhesive Plate Cover Foil	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Sterile disposable tubes and pipette tips
- Centrifuge
- CO_2 incubator
- Microplates with round- and flat-bottoms, such as the Nunc-Immuno MicroWell 96 well plates
- Microplate reader
- Microplate shaker

For the preparation of working solutions

- Double-distilled water
- Tris
- $MgCl_2$
- EDTA
- PBS, sterile
- Exonuclease III
- 2-mercaptoethanol
- Triton X-100
- H_2SO_4
- NaOH
- HCl
- K_2HPO_4
- KH_2PO_4

1.4. Application

The Cellular DNA Fragmentation ELISA is used to determine cell death, a key parameter in the biological studies of a wide variety of cells.

- Measure apoptotic cell death by detection of BrdU-labeled DNA fragments in the cytoplasm of affected cells.
- Measure cell-mediated cytotoxicity by detection of BrdU-labeled DNA fragments released from damaged target cells into the culture supernatant.
- Characterize the type of cell death by performing kinetics and detection of BrdU-labeled DNA fragments in the cytoplasm of apoptotic cells, as well as in the cell culture supernatant released from necrotic cells or at late stages of apoptosis.

i *The ELISA format allows processing of a large number of samples.*

1.5. Preparation Time

Assay Time

Approximately 4.5 to 5.5 hours.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The Cellular DNA Fragmentation ELISA is used with culture supernatant and cytoplasmic lysates of cells containing DNA metabolically prelabeled with BrdU, such as cell lines and other *in vitro* proliferating cells.

Safety Information

The following reagents are toxic or corrosive and must be handled with care:
- 5′-bromo-2′-deoxyuridine
- Tetramethylbenzidine (TMB)
- H_2SO_4
- NaOH
- HCl
- 2-mercaptoethanol

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis/Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Preparation of kit working solutions

Solution	Reconstitution/Preparation of Working Solution	Storage and Stability	For use in...
1	Reconstitute Anti-DNA (Bottle 1) lyophilizate in 1 ml double-distilled water for 10 minutes at +15 to +25°C; mix thoroughly.	Store 6 months at +2 to +8°C.	Solution 3
2	Reconstitute Anti-BrdU-POD (Bottle 2) lyophilizate in 1 ml double-distilled water for 10 minutes at +15 to +25°C; mix thoroughly.	Store 6 months at +2 to +8°C.	Solution 6
3	▪ For 1x solution, dilute 1 ml of 10x Coating buffer (Bottle 3) with 9 ml double-distilled water. ▪ Shortly before use, dilute 0.2 ml of reconstituted Anti DNA (solution 1) with 9.8 ml 1x Coating buffer.	Unstable, prepare immediately before use.	▪ Solution 3 ▪ Coating of microplates.
4	▪ Prewarm 10x Washing buffer (Bottle 4) to +15 to +25°C. ▪ For 1x solution, dilute 40 ml of 10x Washing buffer with 360 ml double-distilled water; mix thoroughly.	Store 2 weeks at +2 to +8°C.	▪ Blocking ▪ ELISA assay ▪ Solution 6
5	▪ Prewarm 2x Incubation buffer (Bottle 5) to +15 to +25°C. ▪ For 1x solution, dilute 20 ml of 2x Incubation buffer with 20 ml double-distilled water; mix thoroughly.	Store 2 weeks at +2 to +8°C	▪ Coating of microplates. ▪ Characterization of cell death. ▪ Measuring apoptosis.
6	Dilute 0.2 ml Anti-BrdU-POD (solution 2) with 9.8 ml 1x Washing buffer (solution 4).	Unstable, prepare immediately before use.	ELISA assay
7	For 1 mM solution, dilute 0.9 ml 1,000x BrdU labeling reagent (Bottle 7) with 8.1 ml sterile PBS or culture medium.	Store 3 months at +2 to +8°C, or several years at −15 to −25°C. ⚠ ***Keep protected from light.***	Batch labeling of cells with BrdU.

Preparation of additional solutions

Solution	Content	Reconstitution/Preparation of Working Solution	Storage and Stability	For use in...
8	Stop solution	Add 560 µl concentrated H_2SO_4 (95 to 97%) to 8 ml ice-cold double-distilled water, mix thoroughly, and dilute up to 10 ml.	Store at least 1 year at +15 to +25°C.	Photometric measurement
9	Exonuclease III solution	▪ Prepare 1x nuclease reaction buffer: 66 mM Tris, 0.66 mM $MgCl_2$, 1 mM 2-mercaptoethanol, pH 8.0. ▪ Dilute exonuclease III with 1x nuclease reaction buffer to a final concentration of 10 U/ml.	Unstable, prepare immediately before use.	ELISA assay
10	1% Triton X-100	For 1x solution, dilute 1 ml 10% Triton X-100 with 8 ml double-distilled water, mix thoroughly, and dilute up to 10 ml.	Unstable, prepare immediately before use.	Positive control protocol.
11	0.25 M NaOH	Dissolve 100 mg NaOH in 8 ml double-distilled water, mix thoroughly, and dilute up to 10 ml.	Unstable, prepare immediately before use.	Positive control protocol.
12	0.25 M HCl	Add 208.4 µl concentrated HCl (37%) to 8 ml ice-cold double-distilled water, mix thoroughly, and dilute up to 10 ml.	Store at least 1 year at +15 to +25°C.	Positive control protocol.
13	0.2 M K_2HPO_4	Dissolve 3.48 g K_2HPO_4 in 80 ml double-distilled water, mix thoroughly, and dilute up to 100 ml.	Store at least 1 year at −15 to −25°C.	Positive control protocol.
14	0.2 M KH_2PO_4	Dissolve 2.72 g KH_2PO_4 in 80 ml double-distilled water, mix thoroughly, and dilute up to 100 ml.	Store at least 1 year at −15 to −25°C.	Positive control protocol.
15	0.2 M K_2HPO_4/ KH_2PO_4, pH 7.0	To prepare solution 15, add solution 14 to solution 13 until adjusted to pH of 7. Adjust pH of solution 13 by adding solution 14 to a pH of 7.0.	Store at least 1 year at −15 to −25°C.	Positive control protocol.

2.2. Protocols

Batch labeling of cells with BrdU

1. Adjust cell number to 2 to 4 × 10^5 cells/ml culture medium.

2. Add BrdU labeling solution (solution 7) to a final concentration of 10 µM.

3. Incubate for 2 hours or overnight at +37°C.

   Labeling time strongly depends on the cell type and the stage of cell culture; average time is 2 to 20 hours.

4. Centrifuge for 10 minutes at 250 × *g*.

5. Carefully and thoroughly remove the BrdU-containing culture medium.

6. Resuspend cells in BrdU-free culture medium.
   – Final concentration: 2 × 10^5 cells/ml for measuring cell-mediated cytotoxicity, or 1 × 10^5 cells/ml for all other applications.

Characterization of cell death

This protocol consists of two parts:
Part 1: The supernatant is analyzed which will contain DNA fragments at early states of necrosis and at late stages of apoptosis.
Part 2: The remaining cells are lysed to release apoptotic DNA fragments located in the cytoplasm.

Part 1

The following steps describe how to sample the supernatant from the labeled cells.

1. Pipette 100 µl of BrdU-labeled cells in culture medium (1 × 10^5 cells/ml from section, **Batch labeling of cells with BrdU**) into duplicate wells of a 96-well, round-bottom microplate.

2. Add 100 µl cell culture medium, containing an appropriate amount of apoptosis inducing reagent per well.

3. Incubate at +37°C in a humidified atmosphere (5% CO_2) for an appropriate period of time, approximately 1 to 6 hours.

4. Centrifuge for 10 minutes at 250 × *g*.

5. Remove 100 µl of the supernatant to analyze in the ELISA assay.

   Store the samples at −15 to −25°C for up to three days.

Part 2

The following steps describe how to continue with the remaining cells to obtain the DNA fragments from the cytoplasm.

1. Carefully and thoroughly remove the remaining supernatant.

2. Add 200 µl 1x incubation solution (solution 5) per well to lyse the cells.
   – Incubate for 30 minutes at +15 to +25°C.

3. Centrifuge for 10 minutes at 250 × *g*.

4. Remove 100 µl of the supernatant to analyze in the ELISA assay.

   Store the samples at −15 to −25°C for up to three days.

Measuring apoptosis

The following steps descibe how to extract apoptotic DNA fragments from the cytoplasm.
Before starting this protocol, see Typical results, **Characterization of the type of cell death***, or use other methods, such as the morphology of the cells or the DNA ladder.*

1. Pipette 100 µl of BrdU-labeled cells in culture medium (1 × 10^5 cells/ml from section, **Batch labeling of cells with BrdU**) into duplicate wells of a 96-well, round-bottom microplate.

2. Add 100 µl cell culture medium containing an appropriate amount of apoptosis-inducing agent per well.

3. Incubate at +37°C in a humidified atmosphere (5% CO_2) for an appropriate period of time, approximately 1 to 6 hours.

4. Centrifuge 10 minutes at 250 × *g*.

5. Carefully and thoroughly remove the supernatant culture medium.

6. Add 200 µl 1x incubation solution (solution 5) per well to lyse the cells.
   – Incubate 30 minutes at +15 to +25°C.

7. Centrifuge 10 minutes at 250 × *g*.

8. Remove 100 µl/well of the supernatant to analyze in the ELISA assay.

   Store the samples at −15 to −25°C for up to three days.

Measuring cell-mediated cytotoxicity

The following steps describe how to extract BrdU-labeled DNA fragments from the supernatant released by dead target cells.

1. Pipette 100 µl BrdU-labeled target cells in culture medium (2 × 10^5 cells/ml from section, **Batch labeling of cells with BrdU**) into duplicate wells of a 96-well, round-bottom microplate.

2. Add 100 µl culture medium containing an appropriate number of effector cells per well. Use a ratio of 0.01 effector cells to 10 target cells.
   – Negative Control: Pipette 100 µl culture medium into different duplicate wells of a 96-well, round-bottom microplate to determine spontaneous release
   of DNA fragments.

3. Incubate for 1 to 6 hours at +37°C in a humidified atmosphere (5% CO_2).

4. Centrifuge 10 minutes at 250 × *g*.

5. Remove 100 µl of the supernatant to analyze in the ELISA assay.

   Store the samples at −15 to −25°C for up to three days.

Positive control

To determine the amount of BrdU incorporated into genomic DNA, it is imperative to denature the full-length DNA for quantitative solubilization. This can be accomplished using one of the following methods.
Method 1: Solubilization of genomic DNA by endogenous nucleases.
Method 2: Solubilization of genomic DNA by NaOH treatment.

Method 1

This method is based on the fact that most cells contain endogenous nucleases. After cell lysis, these nucleases will be activated by Ca^{2+}- and Mg^{2+}-ions contained in the culture medium and will partially solubilize the DNA.

Depending on the cell line, the level of endogenous nucleases will vary and may result in poor fragmentation and subsequent solubilization and therefore, may not be quantitative.

1. Pipette 100 µl of BrdU-labeled cells in culture medium (1 × 10^5 cells/ml from section, **Batch labeling of cells with BrdU**) into a well of a 96-well, round-bottom microplate.

2. Add 100 µl double-distilled water containing 1% Triton X-100 (solution 10).

3. Incubate cells for the same time as for the cellular assay at +37°C, see sections **Characterization of cell death**, **Measuring apoptosis**, and **Measuring cell-mediated cytotoxicity**).

4. Centrifuge 10 minutes at 250 × *g*.

5. Remove 100 µl of supernatant to analyze in the ELISA assay.

Method 2

Genomic DNA is denatured and degraded by NaOH treatment.

DNA solubilization by NaOH differs from all physiological nuclease cleavage during apoptosis, necrosis, and cell-mediated cytotoxicity. It provides a maximal amount of degraded DNA and a maximal value. This artificial method of degradation will not be obtained physiologically.

1. Transfer 500 µl of BrdU-labeled cells in culture medium (1 × 10^5 cells/ml from section, **Batch labeling of cells with BrdU**) to a 1.5 ml reaction tube.

2. Centrifuge 5 minutes at 250 × *g*.

3. Discard supernatant.

4. Add 125 µl 0.25 M NaOH (solution 11).
   – Incubate for 30 minutes at +15 to +25°C.

5. Add 125 µl 0.25 M HCl (solution 12).
   – Add 250 µl 0.2 M K_2HPO_4/KH_2PO_4, pH 7 (solution 15).

6. Centrifuge 5 minutes at 11,000 × *g*.

7. Remove 400 µl of supernatant and titrate in the ELISA assay.

   For dilution, use the incubation solution (solution 5).

Coating of the microplate

The following steps describe coating of the microplate with the Anti-DNA.

1. Pipette 100 µl Anti-DNA coating solution (solution 3) into each well of a 96-well, flat-bottom microplate.

2. If you want to proceed with the assay on the same day, incubate for 1 hour at +37°C.

3. If you want to proceed with the assay on the next day, cover the microplate with a Self-adhesive Plate Cover Foil and incubate overnight at +2 to +8°C.

4. If you want to store the coated microplate for up to 1 week, incubate for 1 hour at +37°C.
   – Remove the coating solution by aspirating.
   – Cover the microplate with an adhesive Cover Foil.
   – Store at +2 to +8°C.
   – Proceed with Step 1 of the **Blocking protocol**.

5. Remove the coating solution by aspirating the buffer.
   – Alternatively, invert the microplate and tap gently on a paper towel.
   – Proceed with the **Blocking protocol**.

Blocking protocol

After the microplate has been coated with the Anti-DNA, nonspecific binding sites are blocked.

1. Add 200 µl of 1x incubation solution (solution 5).
   – Cover the microplate with an adhesive Cover Foil.
   – Incubate 30 minutes at +15 to +25°C.

2. Remove the incubation solution by aspirating or inverting.

3. Wash the wells three times with 250 to 300 µl of washing solution (solution 4) for 2 to 3 minutes each.

4. Remove the washing solution by aspirating or inverting.
   – Proceed to the ELISA Assay.

ELISA assay

The following steps describe how to detect BrdU-labeled DNA fragments in the samples.

1. Transfer 100 µl of a sample obtained in sections **Characterization of cell death**, **Measuring apoptosis**, **Measuring cell-mediated cytotoxicity, and Positive control** into a well of the precoated 96-well, flat-bottom microplate, see section **Coating of the microplate**.

2. Cover the microplate tightly with an adhesive Cover Foil.
   – Incubate 90 minutes at +15 to +25°C or overnight at +2 to +8°C.

3. Remove the solution by aspirating or inverting.

4. Wash the wells three times with 250 to 300 µl washing solution (solution 4) for 2 to 3 minutes per wash.

5. If you want to fix and denature the DNA by microwave irradiation:
   – Leave the washing solution in the well after the last wash step in Step 4.
   – Place the uncovered microplate in a microwave oven.
   – Place a 500 ml beaker containing 300 ml water into the microwave oven.
   – Irradiate for 5 minutes on medium power (500 W).
   – Cool the microplate for approximately 10 minutes at −15 to −25°C.
   – Remove the fluid by aspirating or inverting.

6. If you want to fix and denature the DNA by nuclease treatment:
   – Pipette 100 µl exonuclease III solution (solution 9) per well.
   – Cover the microplate tightly with an adhesive Cover Foil.
   – Incubate for 30 minutes at +37°C.
   – Remove the solution by aspirating or inverting.
   – Wash the plate as described in Step 4.

7. Add 100 µl of Anti-BrdU-POD conjugate solution (solution 6) per well.
   – Cover the microplate tightly with an adhesive Cover Foil.
   – Incubate 90 minutes at +15 to +25°C or overnight at +2 to +8°C.

8. Wash as described in Step 4.
   – Continue with photometric measurement.

Photometric measurement

The values can be measured at either 370 or 450 nm.

Measurement at 450 nm will result in a 2 to 3 fold increase of O.D. values, but does not allow the kinetics of color development to be followed.

1. Pipette 100 µl substrate solution into each microplate well used.

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2. If you want to measure at 450 nm (reference wavelength 690 nm):
   – Incubate in the dark on a microplate shaker until color development is sufficient.
   – Add 25 µl stop solution (solution 8) per well.
   – Incubate 1 minute on a plate shaker.
   – Measure within 5 minutes after adding the stop solution.

   Color will begin to fade after 5 minutes.

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3. If you want to measure at 370 nm (reference wavelength 492 nm):
   – Do not add stop solution.
   – Measure the absorbance at specific time points after substrate solution has been added (i.e., every 30 seconds) to follow the kinetics of color development.

2.3. Parameters

Accuracy

Results correlate to data obtained with the [^{3}H]-thymidine-based DNA fragmentation assay, and the [^{3}H]-thymidine- and [^{51}Cr]-release assays.

Sensitivity

The Cellular DNA Fragmentation ELISA is more sensitive than the [^{3}H]-thymidine-based DNA fragmentation assay and as sensitive as the [^{3}H]-thymidine- and [^{51}Cr]-release assays.

- In apoptosis, the ELISA allows the detection of BrdU-labeled DNA fragments in the cytoplasmic fraction of 1×10^{3} cells/well.
- In cell-mediated cytotoxicity, the ELISA allows the detection of BrdU-labeled fragments in the supernatant of 2×10^{3} target cells/well.

Specificity

- Anti-DNA antibody binds to single- and double-stranded DNA. It does not cross-react with BrdU.
- Anti-BrdU-POD, Fab fragments bind to BrdU incorporated into DNA after denaturation of the DNA. There is no cross-reactivity with other cellular components, such as thymidine or uridine.

3. Results

The following figures show typical results when using this kit to:

- Characterize the type of cell death.
- Measure apoptosis.
- Measure cell-mediated cytotoxicity.

Characterization of type of cell death

The Cellular DNA Fragmentation ELISA enables the measurement of DNA fragments in the cell cytoplasm (lysate) and the culture supernatant (SN). Figure 1 shows that upon increased time of exposure to the apoptosis-inducing agent camptothecin (CAM), DNA fragments appear first in the cell lysate. No BrdU-labeled DNA fragments were detected in the supernatant during the first 4 hours after cell death induction, indicating that DNA fragmentation occurred prior to plasma membrane lysis.

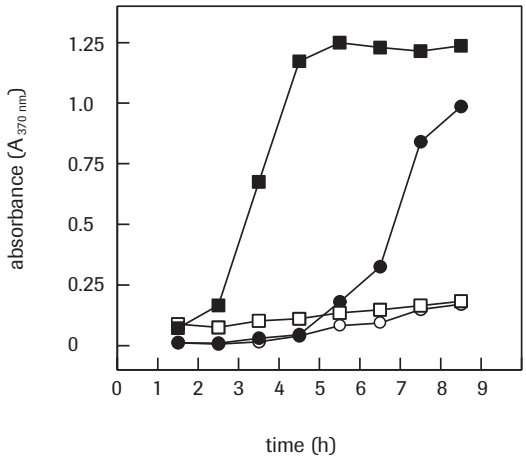

Fig. 1: Kinetics of CAM-induced apoptotic cell death in HL-60 cells. 1 × 10^4 BrdU-labeled cells/well were incubated in either the presence of 200 ng/ml CAM (■, ●), or in the absence of CAM (□, ○) for 1 to 8 hours at +37°C. After the times indicated, 100 µl/well supernatant (●, ○) and 100 µl/well lysate (■, □) were removed and tested by ELISA. **Conclusion:** Cell death due to apoptosis. Necrotic cells would have released DNA fragments into the supernatant at very early stages of cell death.

Measuring apoptosis

Figure 2 shows sensitive detection of nucleosomes in the cytoplasmic fractions at different cell concentrations.

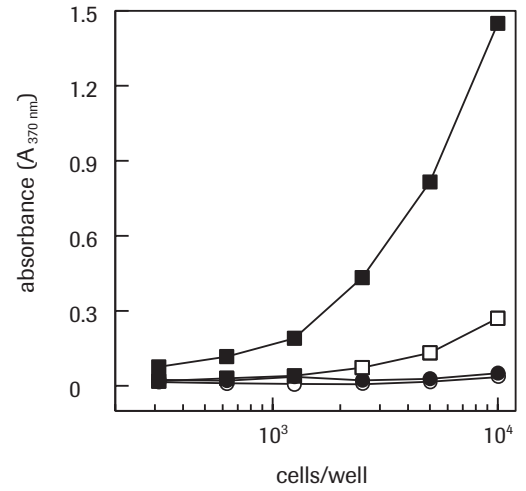

Fig. 2: Measuring apoptosis with the Cellular DNA Fragmentation ELISA. BrdU-labeled HL-60 cells were cultured at different concentrations in the presence of 200 ng/ml CAM (■, ●), or in the absence of CAM (□, ○) for 3 hours at +37°C. After incubation, supernatants (●, ○) and lysates (■, □) were tested by ELISA.

Measuring cell-mediated cytotoxicity

Effects of inducing agent or effector cell concentration can be measured over time. Typical results are shown in Figure 3.

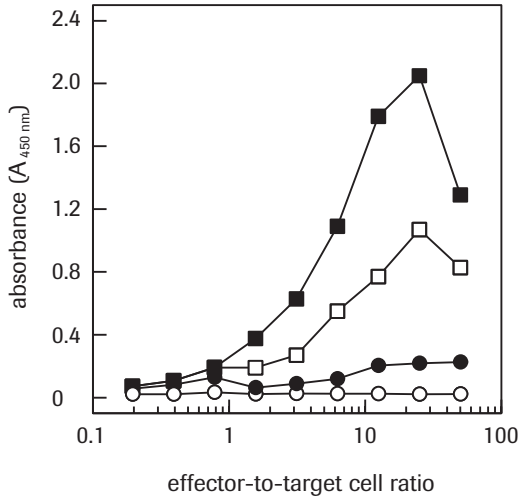

Fig. 3: Kinetics of CTL-mediated cytotoxicity in P815 target cells. 2 × 10^4 BrdU-labled target cells/well were incubated with CTLs at different effector-to-target cell ratios for 1 hour (○), 2 hours (●), 4 hours (□), and 6 hours (■), respectively. After incubation, 100 µl/well supernatant was removed and tested by ELISA.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low absorbance value from positive control, see section **Positive control, Method 2**, solubilization by NaOH.	Doubling time of cell line is >30 hours.	Increase the number of cells/well to 2 to 3 × 10^4.
		Increase labeling time to 24 hours.
	Filter wavelength is not suitable.	See section **Photometric measurement**.
Low signal of positive control, see section **Positive control, Method 1**, solubilization by endogenous nucleases.	Endonuleases require Ca^{2+} and Mg^{2+} for activity.	Add 5 mM Ca^{2+} and 10 mM Mg^{2+}.
	Cell line has low endogenous levels of nuclease.	Use the solubilization by NaOH method, see section **Positive control, Method 2**.
	Microwave irradiation is too high or too low.	Use a different microwave oven.
		Use the solubilization by NaOH method, see section **Positive control, Method 2**.
Low signal of samples and high signal of positive control.	No apoptosis.	Increase concentration of apoptosis-inducing agent.
		Prolong incubation time of apoptosis-inducing reagent.
High signal in untreated samples and high signal of positive control.	Cells died spontaneously.	Check condition of cell culture.
	Cell density is too high.	Reduce number of cells per well.
	Cells have a fast doubling time.	Reduce labeling time.
Low reproducibility between duplicate cultures.	Insufficient lysis of individual cells.	Cells should be completely dispersed and resuspended during lysis step.
	Nuclear DNA pellet was disturbed during supernatant removal after lysis.	Centrifuge with higher speed and carefully remove supernatant.
	Solution in wells evaporating by excessive irradiation.	Use at least 300 ml water in a beaker to absorb the excess energy from the microwave oven and to keep atmosphere humidified.
		Make sure that the washing solution does not boil; check after 2 minutes of irradiation.

5. Additional Information on this Product

5.1. Test Principle

How this product works

The assay is based on a quantitative sandwich enzyme immunoassay principle using two mouse monoclonal antibodies directed against DNA and BrdU (Fig. 4). Cells proliferating *in vitro* are incubated with the nonradioactive thymidine analogue BrdU, which is incorporated into the genomic DNA. BrdU-labeled DNA fragments are released from the cells into the cell cytoplasm during apoptosis, or into the cell culture supernatant during cell-mediated cytotoxicity. These DNA fragments are detected immunologically by the ELISA technique using an anti-DNA antibody bound to the microplate to capture the DNA fragments, and an anti-BrdU antibody-POD conjugate to detect the BrdU contained in the captured and subsequently denatured DNA fragments.

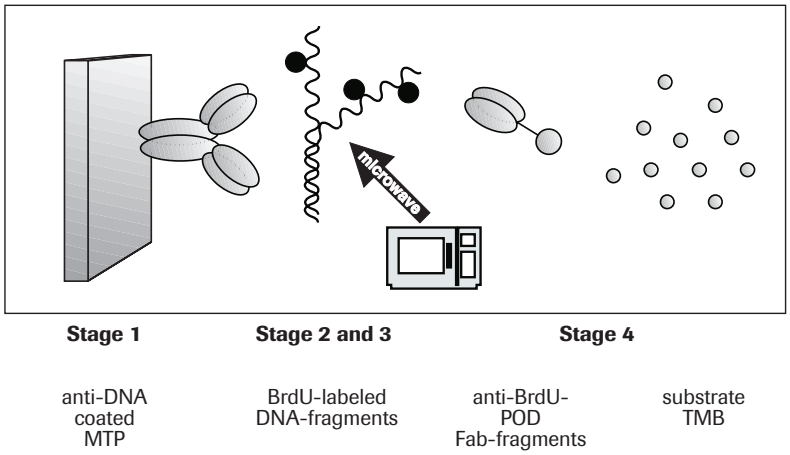

Fig. 4: Test principle

1. Coating of the microplates with anti-DNA antibody and blocking of nonspecific binding sites, see section **Coating of the microplate**[(1)].

2. Labeling of the cells with BrdU, see section **Batch labeling of cells with BrdU**.

3. Additional options are shown in the following table.

If you want to...	then...
Characterize the type of cell death occurring,	set up a kinetics assay and/or look for the appearance of DNA fragments in the culture supernatant as well as in the cytoplasm, see section **Characterization of cell death**[(1)].
Measure apoptosis,	look for appearance of DNA fragments in the cytoplasm only, see section **Measuring apoptosis**[(1)].
Measure cell-mediated cytotoxicity,	look for appearance of DNA fragments in the supernatant released from dead target cells, see section **Measuring cell-mediated cytotoxicity**[(1)].
Perform a positive control,	solubilize the genomic DNA by endogenous nucleases or by NaOH treatment, see section **Positive control**.

4. Determine the quantity of DNA fragments in the sample by ELISA and photometric determination with TMB as substrate, see sections **ELISA assay and Photometric measurement**.

(1) Possible stopping points.

Eukaryotic cell death

Cell death can occur by two different mechanisms: apoptosis and necrosis. Cell-mediated cytotoxicity by cytotoxic T lymphocytes (CTL) and natural killer (NK) cells shows features of both mechanisms.

Apoptosis

Apoptosis or programmed cell death is the most common form of eukaryotic cell death. It is a biological suicide mechanism preserving homeostasis and is essential in many physiological processes, such as embryogenesis, maturation of the immune system, or development of the nervous system.
The main characteristics are:
- Prelytic, nonrandom mono-and oligonucleosomal length fragmentation of DNA ("ladder" pattern after agarose gel electrophoresis).
- Formation of membrane-bound vesicles ("apoptotic bodies").
- Cell shrinkage due to condensation of cytoplasm.

Necrosis

Necrosis is also called pathological cell death because it occurs after cells have been exposed to extreme physiological conditions such as hypothermia, or is evoked by agents such as complement or lytic viruses. *In vivo* necrotic cell death is often associated with extensive tissue damage resulting in an intense inflammatory response.
The main characteristics are:
- Swelling of organelles and of the cells, resulting in cell lysis due to loss of membrane integrity.
- Postlytic DNA fragmentation.
- Random digestion of DNA (DNA smear after agarose gel electrophoresis).

Cell-mediated cytotoxicity

Cells of the immune system, such as CTLs, NKs, or lymphokine-activated killer cells (LAKs) can recognize and destroy damaged, infected, and mutated target cells. Two possible cytocidal mechanisms are involved:
- Apoptosis
- Lytic mechanism by which lytic molecules such as perforin are secreted by the effector cell and polymerize to form lytic pores in the target cell membrane.

The mechanisms are not mutually exclusive, but complementary.
The main characteristic is:
- Fragmented DNA is released from the cytoplasm into the culture supernatant due to pore formation in the target cell plasma membrane.

5.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Editorial changes.
Contents chapter and Storage Conditions chapter: Cap color for bottle 6 updated.

6.3. Trademarks

All product names and trademarks are the property of their respective owners.

6.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

6.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed

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