Technical Data Sheet (TDS)

Product Name: DAD™ Kit

Catalogue Number: BVDK100

BVDK50

BVDK20

1. Product Overview

The Dynamic Apoptosis Detection Kit™ (DAD™ Kit) is an advanced apoptosis detection assay designed to overcome the limitations of conventional assays. A small peptide conjugated with multiple fluorophores, the DAD™ Kit provides sensitive, calcium-independent, and single-step detection of apoptotic cells in both 2D and 3D biological systems. It reliably distinguishes live, early apoptotic, and late apoptotic cells, ensuring high accuracy for cell biology, cancer research, stem cell studies, and organoid cultures.

2. Key Features

- Single-step protocol – simplifies workflow and minimizes handling errors
- Calcium-independent detection – unlike conventional assays, no Ca^{2+} is required
- Non-cell penetrating – enables true live-cell imaging without compromising cell viability
- Highly sensitive detection of early and late apoptosis
- Compatible with in vivo studies, 3D organoids, and stem cell systems
- Validated with flow cytometry and confocal microscopy
- Cost-effective and scalable – available in 20, 50, and 100 test pack sizes
- Available with multiple fluorophores (BODIPY, FITC, IANBD, Cy3, Cy5) for multiplexing

3. Applications

- Apoptosis detection in various cell lines and primary cells
- Live-cell imaging studies
- 3D organoid and spheroid cultures
- Stem cell biology
- Cancer biology and drug screening
- High-throughput imaging and flow cytometry analysis

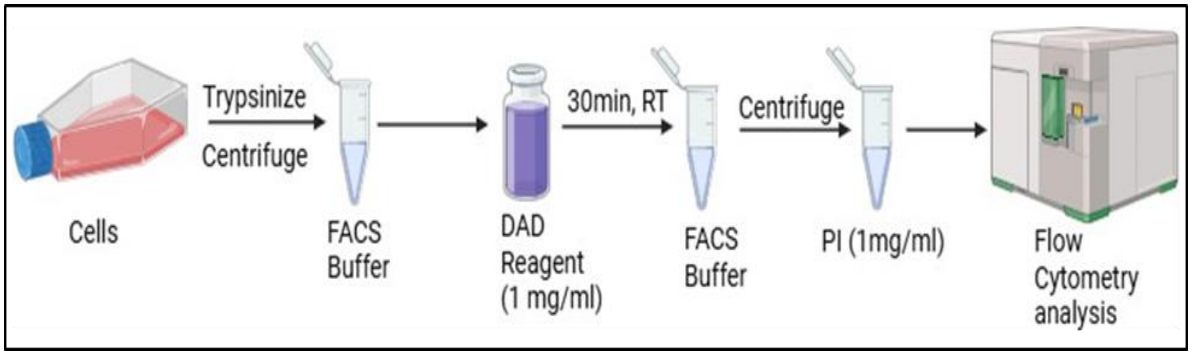
4. Kit Components & Storage

- Each kit contains proprietary fluorescent peptide conjugates, optimized buffers, and user instructions.
- Available fluorophores: BODIPY, FITC, IANBD, Cy3, Cy5
- Storage: Store at -20°C, protected from light
- Shelf Life: 12 months under recommended storage conditions

5. Procedure

A. Flow Cytometry-Based Detection Using DAD™-FITC

- Harvest cells and prepare a cell pellet by centrifugation at 1200 rpm for 5 minutes
- Discard the supernatant and resuspend the pellet in 500 µL of phosphate- buffered saline (PBS)
- Centrifuge again at 1200 rpm for 5 minutes and discard the supernatant
- Resuspend the pellet in 100 µL of FACS buffer
- Add 1 µL of DAD™ (1 mg/mL stock) to the suspension and incubate for 30 minutes at room temperature in the dark
- Following incubation, add 400 µL of FACS buffer and centrifuge at 1200 rpm for 5 minutes at 4 °C
- Discard the supernatant and resuspend the pellet in 100 µL of FACS buffer
- Add 5 µL of propidium iodide (PI, 1 mg/mL stock) and incubate for 5 minutes at room temperature
- Add 400 µL of FACS buffer, mix gently, and proceed to analysis using a flow cytometer



B. Apoptotic Cell Staining for Imaging Using DAD™-FITC

- Culture cells on sterile coverslips under standard growth conditions
- Induce apoptosis using the desired method
- Remove the culture medium and wash the cells twice with PBS
- Add medium containing DAD™ at a final concentration of 8 µM
- Incubate the cells for 1 hour at 37°C in the dark
- Wash the cells twice with PBS
- Fix the cells using 4% paraformaldehyde for 30 minutes at room temperature
- Wash the fixed cells twice with PBS
- Counterstain nuclei with DAPI (1µg/mL) for 5 minutes at room temperature
- Wash twice with PBS and mount the coverslips for imaging
- Acquire fluroscence images using appropriate filters for green (DAD™ -FITC) and blue (DAPI) channels

6. Safety Information

- Classification: Not hazardous under GHS criteria.
- Precautions: Handle according to good laboratory practices.
- First Aid: Rinse with water in case of eye or skin contact; seek medical advice if unwell.
- Disposal: Dispose in accordance with institutional and local regulations.

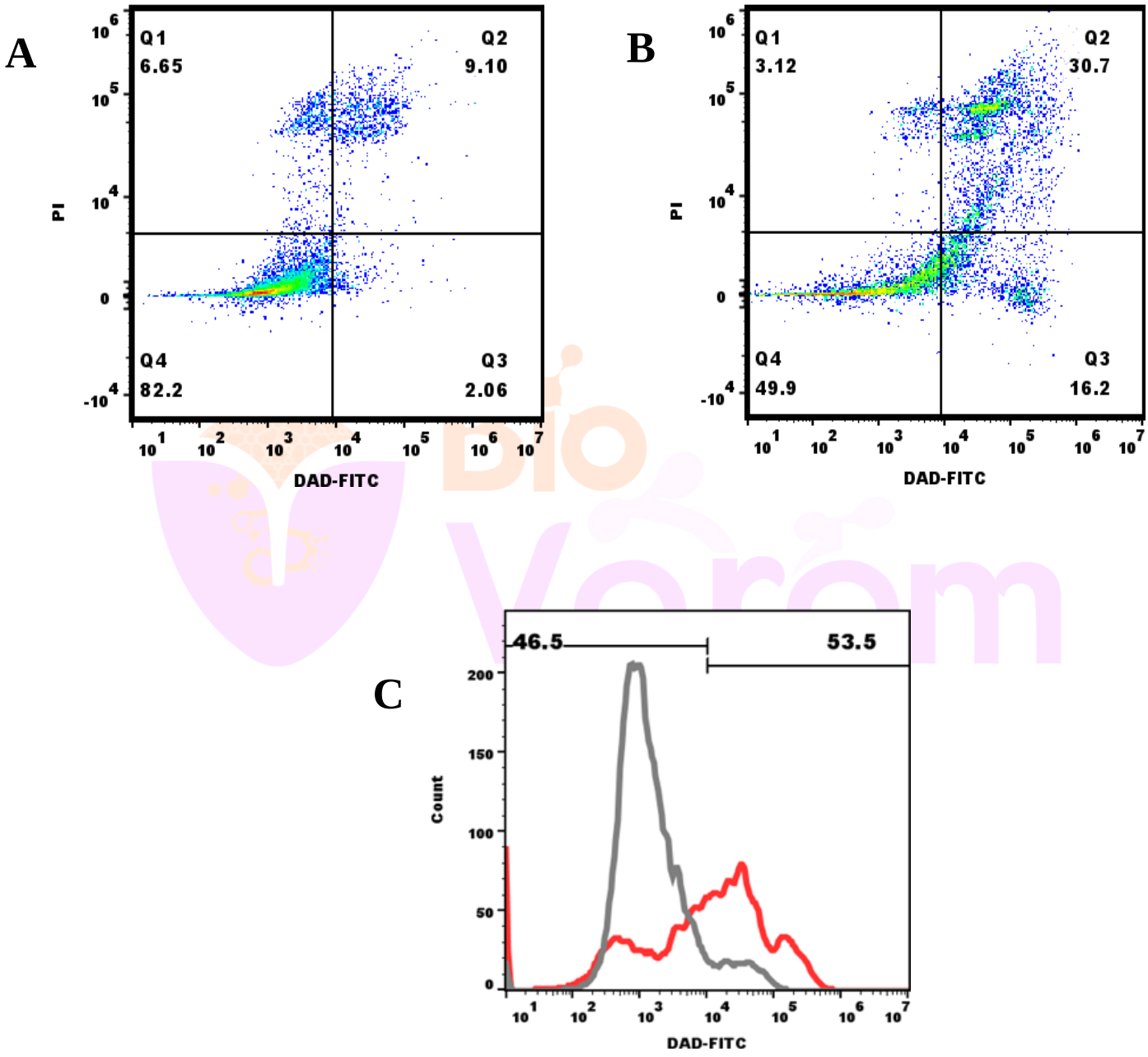
7. Ordering Information

DAD™ Kit is available in 20, 50, and 100 test formats with flexible dye options.

8. References

Gayatri, M.B. *et al.* (2023a) 'AMPK-induced novel phosphorylation of RUNX1 inhibits STAT3 activation and overcome imatinib resistance in chronic myelogenous leukemia (CML) subjects', *Cell Death Discovery*, 9(1). doi:10.1038/s41420-023-01700-x.

9. Product Data



Apoptosis detection in HEK cells using DAD-FITC and Propidium iodide (PI).

A. Untreated control HEK cells. **B.** Staurosporine treated HEK cells. **C.** Histogram overlay represents a rightward fluorescence shift in the apoptotic population (red) compared with control cells grey).

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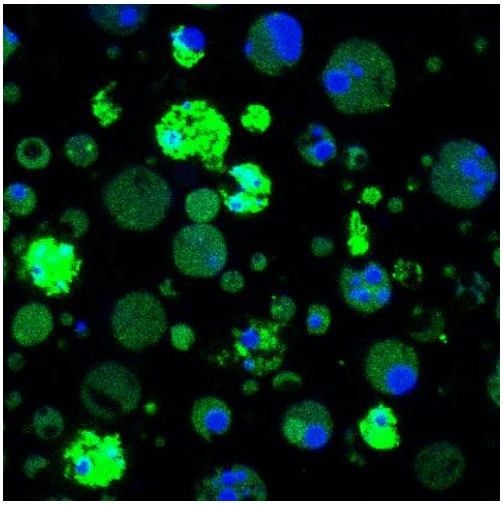

D. iPSC cells treated with 0.5 µM Staurosporine for 3 hrs and then stained with 8 µM DAD-FITC and counterstained with 1 µg/mL DAPI. Apoptotic cells (green) are DAD-FITC positive, and nuclei (blue) are DAPI positive.

10. Contact Information

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For Research Use Only.

Protocol

A. Flow Cytometry-Based Detection Using DAD™-FITC

Start with Cell Pellet

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Resuspend in 500 µL PBS

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Centrifuge @ 1200 rpm, 5 min → Discard supernatant

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Resuspend pellet in 100 µL FACS buffer

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Add 1 µL DAD™ (1 mg/mL); Incubate 30 min, RT, dark

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Add 400 µL FACS buffer; Centrifuge @ 1200 rpm, 5 min, 4 °C → Discard supernatant

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Resuspend pellet in 100 µL FACS buffer

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Add 5 µL Propidium Iodide (1 mg/mL); Incubate 5 min, RT

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Add 400 µL FACS buffer & mix

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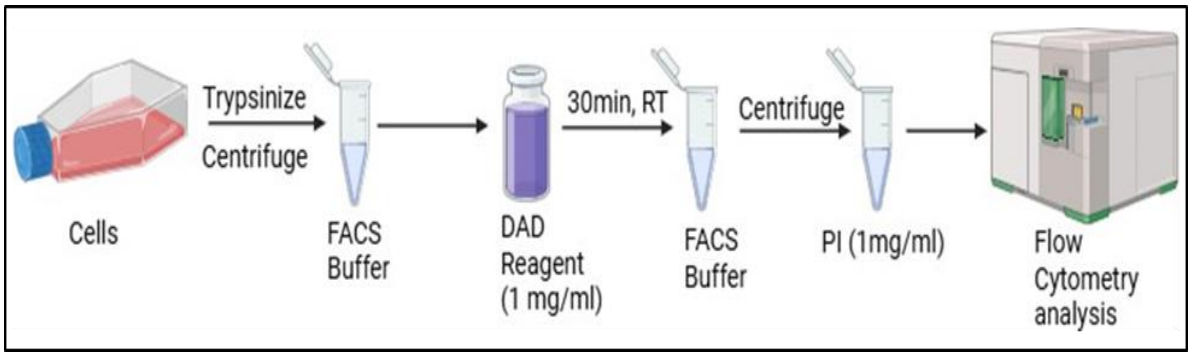
Proceed with Flow Cytometer Analysis



B. Apoptotic Cell Staining for Imaging Using DAD™-FITC

Culture cells on coverslips

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Induce apoptosis by desired method

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Remove medium & wash twice with PBS

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Add DAD™ in medium (final concentration: 8 µM); Incubate 1 hr at 37 °C, dark

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Wash cells twice with PBS

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Fix with 4% paraformaldehyde, 30 min, RT

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Wash cells twice with PBS

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Add DAPI (1 µg/mL); Incubate 5 min, RT

↓

Wash cells twice with PBS

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Image using Green (DAD™-FITC) & Blue (DAPI) channels