Miltenyi Biotec

7-AAD Staining Solution

For *in vitro* diagnostic use

IVD CE

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Str. 68
51429 Bergisch Gladbach
Germany

+49 2204 8306-8484
www.miltenyibiotec.com

1. General information

Intended use
7-AAD Staining Solution is intended for *in vitro* diagnostic use in mono- or multiparametric analyses of human peripheral blood using flow cytometry. It is used for the evaluation of cell viability in the analysis of human leukocytes.

Reagents and contents

Product	Σ 100	Volume	REF
7-AAD Staining Solution	for 100 tests	1 mL	170-080-032

2. Technical data and background information

Components	1 mL 7-AAD Staining Solution
Product formulation	The ready-to-use 7-AAD Staining Solution is supplied in phosphate-buffered saline, pH 7.2, at a concentration of 52.5 µg/mL
+2 °C +8 °C	Store at +2 °C to +8 °C. Do not freeze.
	Store protected from light.
	The use-by date is indicated on the vial label.
	For in-use stability at +2 °C to +8 °C storage temperature refer to the use-by date indicated on the vial label. Do not use the reagent after the use-by date.

Background information
7-AAD (7-amino-actinomycin D) is a fluorescent dye that intercalates into double-stranded DNA (GC rich regions). It is excluded from viable cells but can penetrate cell membranes of dead or dying cells. Therefore, it can be used for the evaluation of cell death and apoptosis. The fluorescence emission maximum for 7-AAD is at 647 nm. When excited at 488 nm, 7-AAD is detected in the red fluorescence channel commonly used for R-phycoerythrin (PE)-CyR5 tandem dye detection, with minimal spectral overlap into the yellow fluorescence channel commonly used for PE detection.

3. Warnings and precautions

- ▲ Analysis results obtained by use of the reagents shall never be the sole basis for classification of disease states.
- ▲ Interpretation of results is under the full responsibility of the user.
- ▲ For all handling, consideration of good laboratory practice (GLP) regulations is recommended.
- ▲ Use of the reagents is restricted to trained and qualified personnel only.
- ▲ All biological specimens and all materials that come into contact with blood and blood products must be treated as infectious material. Regulations for the treatment and disposal of infectious material must be followed.
- ▲ Reagent contains 7-AAD, which in pure form is potentially carcinogenic. Although this mixture is not classified as dangerous at product concentration, it is recommended to avoid contact with skin, mucosa and eyes.
- ▲ For material required but not provided the manufacturers recommendations and safety regulations must be followed.
- ▲ Reagents should not be used if signs of leakage are observed. Use undamaged and sealed vials only.

Directions of the package insert must be followed to obtain accurate and reproducible results.

4. Application

Apoptotic and/ or dead cells are a source of interference in the analysis of viable cells using flow cytometry. Therefore, excluding apoptotic and/or dead cells from flow cytometry data is a critical step to ensure accurate results and analysis. The use of 7-AAD Staining Solution as a viability dye is a recognized benefit in the analysis of total and/or viable haematopoietic progenitor cells.

5. Materials required but not provided

- Disposable capped polystyrene tubes, 12×75 mm
- PEB Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5 % bovine serum albumin (BSA), and 2 mM EDTA by diluting e.g. MACS BSA Stock Solution (Order number 130-091-376) 1:20 with autoMACS Rinsing Solution (Order number 130-091-222). Keep buffer cold (+2 °C to +8 °C).
- Red Blood Cell Lysis Solution: e.g. Red Blood Cell Lysis Solution (10x), IVD, Miltenyi Biotec (Order number 170-080-033), or equivalent
- Double-distilled water
- Micropipettes with disposable tips: variable micropipettes with volume ranges of 10–100 µL and 100–1000 µL
- Low speed centrifuge: minimum speed 300×g, with 12×75 mm tube carriers
- Vortex mixer
- Flow cytometer with appropriate laser and filter settings

6. Protocol

Principle of method:
Incubating a sample of interest, e.g. peripheral blood mononuclear cells, with the provided reagent leads to fluorescent staining of apoptotic and/or dead cells. Analysis of the sample is performed in a flow cytometer at a single-cell level. The analysis is based on the detection of characteristic light emission patterns emitted by the fluorescently labeled cell upon excitation with laser light. The collected data can be processed and analyzed using flow cytometry software.

Important notes:

Exposure of reagents to temperatures below +2 °C and above +8 °C and to light should be minimized during handling.

Sample requirements

- Reagents can be used for determination of antigen positive cells in whole blood samples by flow cytometry. Each cell source can have different storage conditions and limitations that should be considered prior to collection and analysis. For collection of patient samples European and national legislation must be followed.
- Cell count of white blood cells should not exceed 5×10^7 cells/mL.

  Note: If necessary dilute cell sample with PEB buffer.

Protocol for staining

1. Dilute 10× Red Blood Cell Lysis Solution 1:10 with double- distilled water (ddH_2O). For example, dilute 1 mL of 10× Red Blood Cell Lysis Solution with 9 mL of ddH_2O.

   Note: Do not dilute with deionized water. Store prepared 1× Red Blood Cell Lysis Solution at room temperature. Discard unused solution at the end of the day.
2. Add 100 µL of whole blood to a 12×75 mm tube.

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3. Add 10 µL of 7-AAD Staining Solution to 100 µL of cell sample in a 12×75 mm tube.

   Optional: Add the recommended amount of fluorochrome-conjugated antibody.

4. Mix well and incubate for 15 minutes in the dark at room temperature (+20 °C to +25 °C).

   Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

5. Add 2 mL of 1× Red Blood Cell Lysis Solution to each tube. Immediately vortex thoroughly for 3 seconds and incubate for 10 to 20 minutes at room temperature in the dark.

6. Centrifuge at 300×g for 10 minutes. Remove supernatant.

7. Wash cells by adding 1–2 mL of buffer, centrifuge at 300×g for 10 minutes. Remove supernatant.

8. Resuspend cell pellet in a suitable amount of buffer and proceed to flow cytometric analysis. Store samples at +2 °C to +8 °C until analysis.

   Note: Minimize exposure of samples to light.

Quality control
It is recommended to run regularly a control sample from a normal adult specimen or commercially available whole blood control as a quality control of the system.

7. Performance characteristics

Precision
7-AAD Staining Solution was tested by flow cytometry using a lyse-wash protocol on whole blood from healthy donors. Reproducibility was assessed by measuring the frequency of apoptotic and/or dead cells in replicate measurements performed by different operators using the same set of different donor samples. Precision was inferred from calculating the mean, standard deviation and coefficient of variation of the frequency of positive cells. All values were within the acceptance criterion.

Analytical specificity
7-AAD is a well-documented viability marker, which binds specifically to cytosine and guanine bases of the DNA double strand of non-viable cells. Combined with its spectral properties it is well-suited for flow cytometry analysis. The results have been published and are referenced in Gill, J.E., Jotz, M.M., Young, S.G., Modest, E.J., Sengupta, S.K., "7-Amino-actinomycin D as a cytochemical probe. I. Spectral properties", 1975, J. Histochem. Cytochem., 23, 793-799 and Schmid, I. *et al.* (1992) "Dead cell discrimination with 7-amino-actinomycin D in combination with dual color immunofluorescence in single laser flow cytometry". Cytometry 13 (2): 204–208 and Keeney, M. *et. al* "Single Platform Flow Cytometric Absolute CD341 Cell Counts Based on the ISHAGE Guidelines" 1998 Wiley-Liss Inc.

8. Limitations

Reagent data performance was collected typically with EDTA-treated blood following a lyse-wash protocol afterwards.
Reagent performance can be affected by the use of other anticoagulants.

9. References

1. Gill, J.E., Jotz, M.M., Young, S.G., Modest, E.J., Sengupta, S.K., "7-Amino-actinomycin D as a cytochemical probe. I. Spectral properties", 1975, J. Histochem. Cytochem., 23, 793-799.
2. Schmid, I. *et al.* (1992) Dead cell discrimination with 7-amino-actinomycin D in combination with dual color immunofluorescence in single laser flow cytometry. Cytometry 13 (2): 204–208
3. Keeney, M. *et. al* "Single Platform Flow Cytometric Absolute CD341 Cell Counts Based on the ISHAGE Guidelines" 1998 Wiley-Liss Inc
4. Clinical and Laboratory Standards Institute (CLSI). Clinical Flow Cytometry Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline - Second Edition . CLSI document H43-A2 (ISBN 1-56238-635-2) 2007
5. Clinical and Laboratory Standards Institute (CLSI). Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline - Second Edition . CLSI document H42-A2 (ISBN 1-56238-640-9) 2007

10. Glossary of symbols

	Manufacturer		In-use stability
REF	Order number	+2°C +8°C	Temperature limit
P/N	Part number		Protect from sunlight
LOT	Batch code	CE	European conformity approval
IVD	*In vitro* diagnostic medical device		Phone
	Use-by date		Website
	Consult instruction for use		