7-AAD
Viability Dye

REF A07704
150 tests ; 3 mL
20 µL / test

Viability Dye

IVD CE

ENGLISH	Specifications 7-AAD Viability Dye
Formulation	Liquid
Volume	3 mL
Calibration	Ready for use
λ excitation	488 nm
Emission peak	655 nm (complex with double strand DNA)

USE
7-AAD Viability Dye is a chemical coloring agent in solution, which is designed for mono- or multiparametric analysis using flow cytometry. It permits non-viable cells to be identified and to be excluded from (viable) cells of interest in the analysis of human leucocytes.

PRINCIPLE
This test depends upon the ability of 7-Amino-Actinomycin D (7-AAD) to insert itself between the tops of successive Cytosine (C) / Guanine (G) bases of the DNA double strand, when the interior of the cell and the nuclear chromatin are accessible.
Staining of the DNA double strand is undertaken by incubating the sample with the 7-AAD Viability Dye. The red cells are then removed by lysis and the leucocytes are analyzed by flow cytometry.
The flow cytometer analyzes light diffusion and the fluorescence of cells. It makes possible the localization of cells within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light (Side Scatter or SS) and the fluorescence detected using a 620 or 675 nm “band pass” filter, corresponding to 7-AAD staining. Other histograms combining two of the different parameters available on the cytometer are also used in the gating stage.
The fluorescence of cells thus gated is analyzed in order to exclude the positively stained events (non-viable) from the unstained ones (viable). The results can be expressed as a percentage of non-fluorescent events in relation to all the events taken into account by the gating.

EXAMPLES OF CLINICAL APPLICATIONS
Apoptotic necrotic and/or damaged cells are a source of interference in the analysis of viable cells using flow cytometry. Non-viable cells can be characterized and identified as they are stained by 7-AAD, whilst living cells, retaining their membranous integrity, are impermeable to 7-AAD and are unstained (i.e., they are 7-AAD negative) (1).
The use of 7-AAD as a viability marker is a well documented benefit in the characterization and numeration of total and/or viable haematopoietic progenitor cells (5).

STORAGE AND STABILITY
The 7-AAD Viability Dye must be kept at between 2 and 8°C and protected from light, before and after the vial has been opened.
Stability of closed vial: see expiry date on vial.
Stability of opened vial: the reagent is stable for 60 days.

EVIDENCE OF DETERIORATION
In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or use the following e-mail address :
immuno-techsup@beckmancoulter.com

PRECAUTIONS
1. Do not use the reagent beyond the expiry date.
2. Do not freeze.
3. Let it come to room temperature (18 – 25°C) before use.
4. Minimize exposure to light.
5. Avoid microbial contamination of the reagents, or false results may occur.
6. This ready for use reagent contains 0.005% (weight / volume) of 7-AAD.
   In pure form, 7-AAD is potentially carcinogenic. Though extremely diluted in the present formulation, this constituent may retain all or part of its harmful effects. It is therefore advisable to avoid all contact with the skin, mucosa and eyes.
   Use this reagent following the precautions for use (notably: the wearing of gloves, gown and protective glasses).
7. This ready-for-use reagent contains 1% (volume / volume) of Dimethyl Sulfoxide (DMSO).
   In pure form, DMSO is an irritant. Though extremely diluted in the present formulation, this constituent may retain all or part of its harmful effects. It is therefore advisable to avoid all contact with the skin, mucosa and eyes.
   Use this reagent following the precautions for use (notably: the wearing of gloves, gown and protective glasses).
8. Never pipette by mouth.
9. 7-AAD is a chromophore subject to degradation by exposure to light. It is packaged in a vial which is opaque to the wavelengths to which it is sensitive, so protecting it during storage.
   Avoid continuous exposure to light during the incubation stages and reduce light exposure of the samples once staining has been undertaken.
10. All blood samples must be considered as potentially infectious and must be handled with care (in particular: the wearing of protective gloves, gowns and goggles). Never pipette by mouth.
11. Blood tubes and disposable material used for handling should be disposed of in ad hoc containers intended for incineration.

SAMPLES
Venous blood must be taken using sterile tubes containing an EDTA salt as the anticoagulant.
The samples should be kept at room temperature (18 – 25°C) and not shaken. The sample should be homogenized by gentle agitation prior to taking the test sample.
The samples must be analyzed within 24 hours of venipuncture.

METHODOLOGY
NECESSARY MATERIAL NOT SUPPLIED
- Sampling tubes and material necessary for sampling.
- Automatic pipettes with disposable tips for 20, 100 and 500 µL.
- Plastic haemolysis tubes.
- Calibration beads: Flow-Set Fluorospheres (Ref. 6607007).
- Red cell lysis reagent. For example: IOTest 3 Lysing Solution (Ref. A07799).
- Buffer (PBS: 0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2).
- Centrifuge.
- Automatic agitator (Vortex type).
- Flow cytometer.

PREPARATION OF SAMPLES
The concentration of leucocytes in the sample must be less than 10^4 cells / µL (10^{10}/L). If necessary, dilute in PBS to bring the leucocyte concentration to 5 x 10^3 cells / µL (5 x 10^9/L).
The procedure uses 100 µL of a sample, tube prediluted or otherwise.

NB:
- Adjustment of the intensity of fluorescence corresponding to 7-AAD can be undertaken using the staining of a sample of whole fresh blood and of non-viable stabilized cells in the same tube (see image in appendix). Under these conditions, the voltage corresponding to the detection of 7-AAD must be adjusted such that viable events (i.e., whole fresh blood) appear on the first decade of an SS histogram, depending on the 7-AAD (see example in the appendix).
- Do not use reagents containing permeating or fixating agents during the procedure in order to prevent artifactually positive staining.

PROCEDURE
1. Add 20 µL of the 7-AAD Viability Dye solution to each test tube.
2. Add 100 µL of the test sample (i.e., the equivalent of approximately 5 x 10^5 cells). Vortex the tubes gently.
3. Incubate for 15 to 20 minutes at room temperature (18 – 25°C), protected from light.

4. Then perform lysis of the red cells, if necessary, by following the recommendations of the lysis reagent used.
   By way of example, if one wishes to use the IOTest 3 Lysing Solution (Ref. A07799) add 2 mL of the working solution (1X), vortex immediately and incubate for 10 minutes at room temperature, protected from light.
   If the sample does not contain red cells, do not undertake the lysis stage, but add 0.5 to 1 mL of PBS.
5. Preparations should be analyzed within 1 hour.

NOTE: In all cases, keep the preparations between 2 and 8°C and protected from light.

PERFORMANCE

SPECIFICITY

Actinomycins are the active biological constituents of a chromophore (2-Amino-4,6 Dimethylphenoxazone-3) and of cyclic pentapeptides (3). They are antibiotics of bacterial origin characterized historically in soil Ascomycetes. The actinomycins form stable complexes with double strand deoxyribonucleic acid (DNA), but do not form this type of complex either with double strand ribonucleic acid (RNA) or with RNA-DNA hybrids, or with single strand DNA or RNA.

7-Amino-Actinomycin D (7-AAD) is an analogue of Actinomycin containing an amine group substituted in position 7 of the chromophore. 7-AAD inserts itself between the tops of the Cytosine / Guanine bases of DNA (4).

The spectral properties of 7-AAD make it a compound which is particularly well suited to flow cytometry analysis (3). The maximum absorption of the 7-AAD / DNA complex is compatible with the blue excitation wavelength of 488 nm for cytometers fitted with an argon laser (2). The peak fluorescence emission in the deep red band (635 to 675 nm) of the 7-AAD / DNA complex permits optimal use of this probe when it is combined with Fluorescein Isothiocyanate conjugated antibodies (FITC) and with R Phycoerythrin (PE) (3). Indeed, in contrast to Propidium Iodide (PI), which is another fluorescent probe used as a DNA marker, the 7-AAD / DNA complex has a reduced overlapping emission spectrum with FITC and PE.

LINEARITY

To test the linearity of staining of this reagent, two cell types - Stem-Trol Control Cells (stabilized control cells, 7-AAD positive) and KG-1a cell line in culture (fresh line, ie 7-AAD negative) - were mixed in different proportions with a constant final number of cells, so that the Stem-Trol Control Cells / KG-1a ratio of the mixture ranged from 0 to 100%.

Aliquots were stained using the procedure described above and linear regression between the expected values and the observed values was calculated.

Specificity	Linear regression	Linearity (R^2)
7-AAD	Y = 1.0278 X + 4.1364	0.9704

EXPECTED VALUES

Each laboratory must compile a list of reference values based upon a group of healthy donors from the local population. This must be done by taking age, sex and ethnic group into account, as well as any other potential regional differences.

In our laboratories, the less-than-6-hour whole blood samples of 10 healthy adults were treated using the reagent described above. The results obtained for the numeration of the events of interest with this reagent (dead leucocytes, ie 7-AAD positive) are given in the table below:

Positive Target Leucocytes	Number	Mean (%)	SD	CV (%)
7-AAD^+	10	7.67	7.54	98

INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 independent measurements of the percentage of staining of a positive target (stabilized Stem-Trol Control Cells, 7-AAD positive) were carried out on a sample of whole fresh blood with positive target cells. The results obtained are summarized in the following table:

Positive Target Stem-Trol	Number	Mean (%)	SD	CV (%)
7-AAD^+	12	3.63	0.55	15.2

LIMITATIONS OF THE TECHNIQUE

1. Flow cytometry may produce false results if the cytometer has not been aligned perfectly, if fluorescence leaks have not been correctly compensated for and if the regions have not been carefully positioned.
2. It is preferable to use a RBC lysis technique with a washing step as this reagent has not been optimized for "no wash" lysis techniques.
3. Accurate and reproducible results will be obtained as long as the procedures used are in accordance with the technical insert leaflet and compatible with good laboratory practices.
4. 7-AAD, the active substance in this reagent, has been optimized so as to offer the best specific/non-specific signal ratio. Therefore, it is important to adhere to the reagent volume/sample volume ratio in every test.
5. In the case of a hyperleucocytosis, dilute the blood in PBS so as to obtain a value of approximately 5 x 10^9 leucocytes/L.
6. In certain disease states, such as severe renal failure or haemoglobinopathies, lysis of red cells may be slow, incomplete or even impossible. In this case, it is advisable to isolate mononucleated cells using a density gradient (Ficoll, for example) prior to staining.

MISCELLANEOUS

See the Appendix for examples and references.

TRADEMARKS

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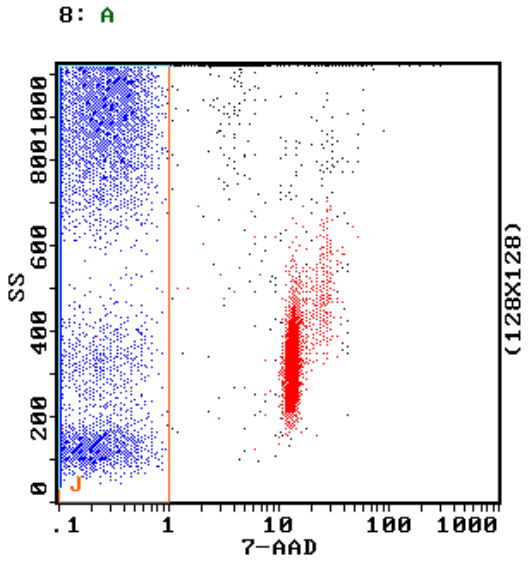
APPENDIX TO REF A07704

EXAMPLES

The graph below is a biparametric representation (Fluorescence Intensity *vs.* Side Scatter) of a lyzed fresh whole blood sample from an healthy donor spiked with Stem-Trol Control Cells (Ref. IM3632). Since Stem-Trol Control Cells are stabilized cells, they are not viable and are stained with the the 7-AAD Viability Dye (visible in the right part of the histogram). In contrast, a majority of the fresh whole blood cells are not stained (visible in the left part of the histogram).



Staining is with the 7-AAD Viability Dye (Ref. A07704) alone. All events are shown. Acquisition and analysis are performed with a COULTER EPICS XL flow cytometer equipped with System II software.

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