

Alkaline Phosphatase Live Stain (500X)

Catalog no. A14353

Size: 50 µL

Store at -20°C

Publication Part Number A14353PPS

MAN0006110

Revision Date 12 December 2011

Description

Alkaline phosphatase (AP) is a phenotypic marker of pluripotent stem cells (PSCs), including undifferentiated embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and embryonic germ cells (EGCs).¹⁻⁴ While AP is expressed in most cell types, its expression is highly elevated in PSCs. AP staining has therefore been used to differentially stain PSCs to easily distinguish them from mouse embryonic fibroblasts (MEF) used as feeders and parental fibroblasts commonly used in reprogramming experiments.^{5,6} However, current available alkaline phosphatase substrates are toxic to the cells, which prevent them from propagating once stained.

The Alkaline Phosphatase Live Stain can be applied to adherent PSCs in culture without loss of proliferation or pluripotency. Staining is specific to PSCs with minimal background in MEF feeders, primary fibroblast cells, and other somatic cell types commonly used for reprogramming, providing an easy-to-use, live monitoring method to track cells during reprogramming or during routine culture of ESC and iPSCs. After removal of dye from the media, fluorescently labeled cells lose their signal within 60–90 minutes.

Note: In general, AP is not an ideal marker to distinguish between undifferentiated and early differentiating cells. Therefore, the AP Live Stain is not meant for distinguishing undifferentiated cells from differentiated cells.⁷

Product Use

For research use only. CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

Features

Alkaline Phosphatase (AP) Live Stain has the following features and benefits:

- Differentially stains PSCs
- Produces similar staining pattern and has similar specificity as other AP dyes
- Unlike other AP dyes, maintains the integrity of the cells

Thawing Alkaline Phosphatase Live Stain

Remove the AP Live Stain vial from the -20°C freezer and thaw at room temperature.

Avoid repeated freeze/thaw cycles and aliquot the AP Live Stain into smaller volumes if necessary.

Preparing Staining Solution

To prepare a 1X AP Live Stain working solution, dilute the 500X stock solution in DMEM/F-12 (Cat. no. 10565-018) as described in Table 1. Use the diluted dye immediately.

Note: It is important to dilute AP Live Stain stock solution in basal media such as DMEM/F-12.

Table 1 Recommended volumes for diluting the AP Live Stain stock solution (500X)

Culture Area	AP Live Stain (500X)	DMEM/F-12
2 cm ²	1 µL	0.5 mL
10 cm ²	3 µL	1.5 mL
20 cm ²	6 µL	3 mL
60 cm ²	12 µL	6 mL

Staining Procedure

1. Remove the growth medium from the cultures to be stained with AP Live Stain.
2. Wash the culture with pre-warmed DMEM/F-12 for 2–3 minutes. Aspirate and repeat.
3. Prepare a 1X AP Live Stain working solution by diluting the 500X stock solution in DMEM/F-12 (see Preparing Staining Solution). Apply an appropriate amount of the 1X AP Live Stain solution directly on to the adherent cell culture.
4. Incubate culture for 20–30 minutes.

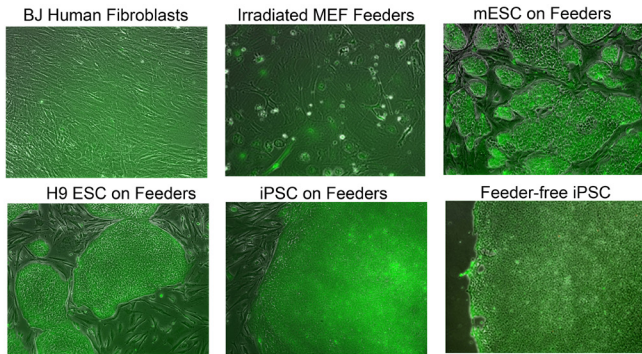
Note: Incubation with media containing FBS, KnockOut™ SR or feeder-free supplements designed for the growth and maintenance of ESCs and iPSCs can interfere with the AP live staining reaction.

5. Remove the AP Live Stain and wash **twice** with DMEM/F-12 for 5 minutes per wash.
Note: This step removes the excess AP Live Stain and reduces the background signal. Handle the cells aseptically, and carefully add and remove the media with minimal disruption to the adherent cells during this step to avoid damage to cells.
6. Following the final wash, add fresh DMEM/F-12 prior to the visualization of fluorescent-labeled colonies under fluorescent microscopy using a standard FITC filter.
Note: Media containing FBS, KnockOut™ SR or feeder-free supplements designed for the growth and maintenance of ESCs and iPSCs can interfere with fluorescent visualization.
7. Images can be captured within 30–90 minutes of staining and the most robust fluorescent colonies can be marked for selection and expansion.
8. Following visualization, replace DMEM/F-12 with fresh growth medium. Selected colonies can be either manually picked or returned to the normal culture conditions.
9. Stained colonies may be restained as early as 24 hours after the initial staining with the AP Live Stain.

Expected Results

The images below show various cells stained with the AP Live Stain following the protocol described on page 1. The differential staining of pluripotent cells easily distinguishes them from the feeder cells on which they have been cultured.

Figure 1 Specificity of the AP Live Stain



Troubleshooting

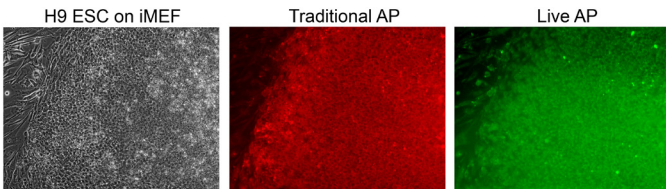
No signal observed

Be sure to use DMEM/F-12 medium to dilute the AP Live Stain and during wash steps.

High background staining in MEFs

Since AP Live Stain depends on differential expression of alkaline phosphatase, dim staining of MEFs may be observed. This pattern is consistent with that observed with other AP dyes (see images below).

Figure 2 Traditional AP dyes versus the AP Live Stain



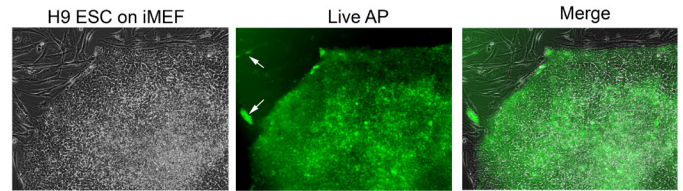
- **Wash steps are critical** to remove the excess dye and to eliminate background staining. To further decrease background staining, perform 3X washes of 5 minutes each for a total of 15 minutes.
- Depending on the sensitivity of the imaging device and the background observed, AP Live Stain concentrations ranging between 0.2–1X can be used, and exposure to AP Live Stain can be reduced to 10 minutes or increased to 40 minutes.

Troubleshooting, continued

High staining seen in a few fibroblast-like cells

Sometimes cells that have a distinct fibroblast morphology may show robust staining with the AP Live Stain. These AP-positive fibroblast-like cells are seen only in ESC-MEF co-cultures and never in the MEF cultures alone (see image below). Observe the entire dish to determine if the robust staining in fibroblast-like structures is in a small fraction of cells.

Figure 3 Staining of H9 ESCs and MEF feeder layers



Survival of cells is low

It is important to handle the cells during staining and subsequent washes carefully. When cells are handled with care during staining and subsequent washing steps, AP Live Stain has been experimentally confirmed not to alter cell survival.

References

1. Andrews *et al.* (1984) *Hybridoma* 3, 33–39.
2. Pera *et al.* (2000) *J Cell Sci* 113, 5–10.
3. Shambloft *et al.* (1988) *Proc Natl Acad Sci USA* 95, 13726–13731.
4. O'Connor *et al.* (2008) *Stem Cells* 26, 1109–1116.
5. Thomson *et al.* (1998) *Science* 282, 1145–1147.
6. Takahashi *et al.* (2007) *Cell* 131, 861–872.
7. Ramirez *et al.* (2011) *Stem Cells* 29, 1469–1474.

Technical Support

For additional product and technical information, such as Safety Data Sheets (SDS), Certificates of Analysis, etc., visit our website at www.lifetechnologies.com. For further assistance, email our Technical Support team at techsupport@lifetech.com.

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