TUNEL staining and the TUNEL assay

Understand the methods used for TUNEL staining (also known as the TUNEL assay), and review TUNEL staining kits

TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) staining, also called the TUNEL assay, detects the DNA breaks formed when DNA fragmentation occurs in the last phase of apoptosis.

Principles of TUNEL staining / the TUNEL assay

The TUNEL staining / TUNEL assay method relies on the enzyme terminal deoxynucleotide transferase (TdT), which attaches deoxynucleotides to the 3'-hydroxyl terminus of DNA breaks. TdT is expressed in certain immune cells and acts during V(D)J recombination – the process that generates antibody diversity.

In TUNEL staining, the nucleotides attached by TdT are tagged either directly with a fluorescent label or with a chemical label that can be indirectly linked to either a fluorescent label or an enzyme.

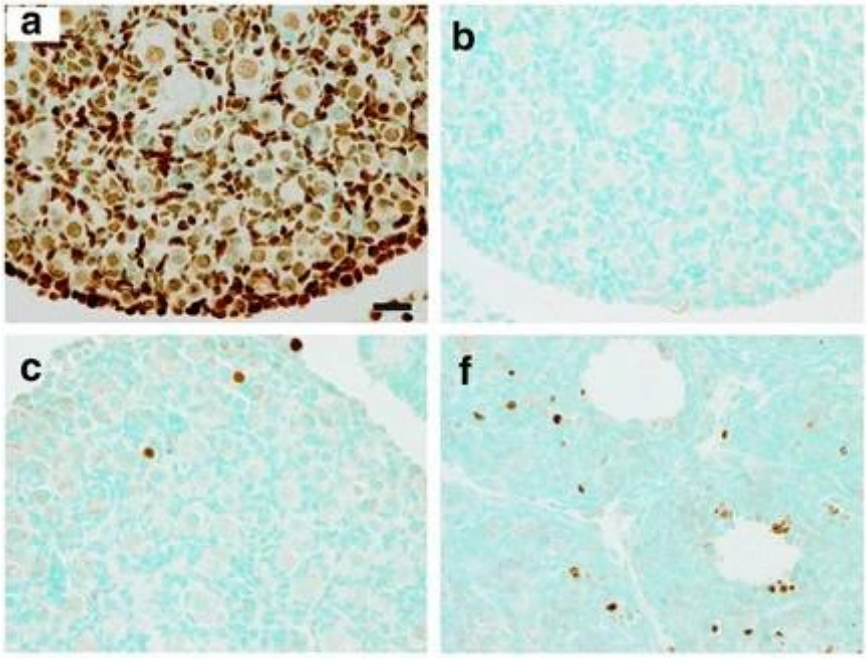
TUNEL staining is a modern alternative to analyzing the formation of DNA fragments during apoptosis using agarose gel electrophoresis.

TUNEL assay methods

TUNEL staining / the TUNEL assay is most commonly analyzed by light microscopy. Fluorescent TUNEL staining / TUNEL assay methods are also suitable for analysis by flow cytometry.

The most commonly used methods for TUNEL staining / the TUNEL assay use

1. a nucleotide directly conjugated to a fluorescent dye (usually FITC)
2. a biotin-tagged nucleotide that is then bound by streptavidin-HRP and detected using a chromogenic HRP substrate, such as DAB, to generate a brown color
3. a bromo-, digoxygenin-, or FITC-tagged nucleotide that is then bound by an antibody specific for that tag. The antibody is labeled with either a fluorescent dye or an enzyme such as HRP for chromogenic detection



Gao Y et al. used HRP-DAB TUNEL to analyze tissue sections from mouse ovaries.

***a**. Section treated with DNase I as positive control.*

***b**. Negative control without TdT enzyme.*

***c** and **f**. representative experimental images. Nuclei stained with the TUNEL assay are brown. Sections were counterstained with Methyl Green.*

Advantages and disadvantages of TUNEL staining methods

TUNEL staining / TUNEL assay protocols that use a nucleotide directly tagged with a fluorescent dye are faster than indirect methods, which use either an antibody or a streptavidin-biotin complex, as they require less staining steps.

Methods that rely on biotin-tagged nucleotides can benefit from the amplification of the streptavidin-biotin complex. They do require additional blocking steps to neutralize endogenous biotin and reduce background staining, although these are common to all researchers running traditional chromogenic immunohistochemistry.

BrdU-based methods can also produce a brighter signal as BrdU is typically more easily incorporated by the TdT enzyme.

The relative popularity of TUNEL staining/TUNEL assay methods

A survey of 50 research papers published in 2017 containing the term “TUNEL Assay” or “TUNEL Staining”, revealed that:

50% of papers used dUTP directly conjugated to FITC

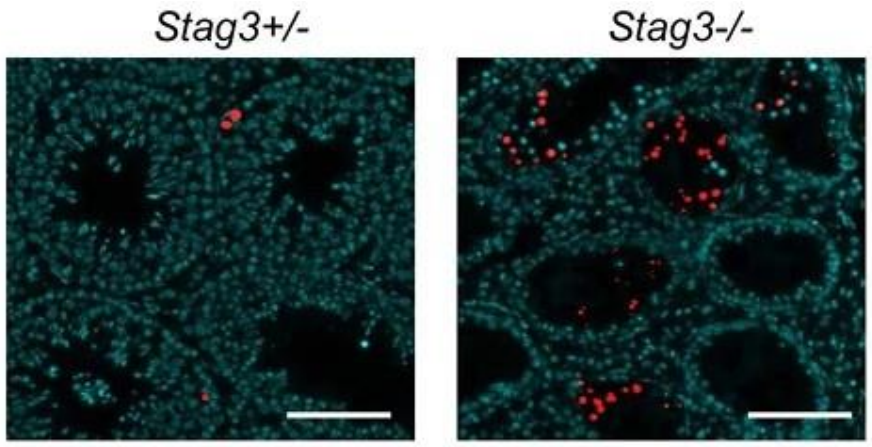
15% used biotin-dUTP and streptavidin-HRP

15% used FITC-dUTP and an anti-FITC antibody conjugated to HRP

12% used digoxygenin-dUTP, and an anti-digoxygenin antibody conjugated to either a fluorescent dye or HRP

8% used Br-dUTP and an anti-BrdU antibody conjugated to a fluorescent dye

All the papers surveyed used the TUNEL assay in imaging rather than flow cytometry. Where HRP was used, DAB substrate was added to create a brown stain. More than 90% of the research papers used a kit for TUNEL staining.



Hopkins J et al used BrdU-Red TUNEL Assay Kit to examine apoptosis in testis from 8-week old Stag3$^{+/-}$ and Stag3$^{-/-}$ mice. Apoptotic cells are red. DAPI was used as a counterstain.

In summary, TUNEL staining provides a useful method for the analysis of DNA fragmentation in apoptosis.