TUNEL assay

(terminal dUTP nick-end labeling to detect apoptotic cells)

REAGENTS:

• ChromaTide™ Alexa Fluor® 488-5-dUTP [1 mM in TE buffer]
Molecular Probes #C-11397 (25 µL => enough for 100 reactions)

•TdT terminal transferase kit (500 U)
Roche #220582

•VECTASHIELD® Mounting Medium and VECTASHIELD® Mounting Medium with DAPI (10ml)
Vector Laboratories #H-1000/#H-1200

PROTOCOL:

•Deparaffinize and rehydrate slides:

3 x 3´	Xylene
3 x 2´	100% ethanol
1 x 2´	95%, 80%, 70% ethanol (each)
1 x 5´	1x PBS

•Microwave antigen retrieval:
4 x 5´ in microwave (600 ml of 10mM NaCitrate, pH 6)
Cool 20´. Wash 3 x 5´ in water. Wash 1 x 5´ in 1x PBS (Phosphate-Buffered Saline).

•Shake off/wipe off excess PBS and circle all sections with ImmunoEdge or PAP pen.

•Block 10 minutes in Equilibration Buffer at room temperature (50 µl/section).

•Add 50 µl of Reaction Buffer to each section and incubate at 37°C for 60-90 minutes.
[One section WITH TdT enzyme, one section without enzyme as a negative control]

•Soak slides in 1xSSC for 15 minutes at room temp to stop reaction.

•Wash 5 x 5´ in 1x PBS.

•Mount the sections in 3:1 Vectashield:DAPI. Coverslip and seal with clear nailpolish.

BUFFERS:

•Equilibration Buffer:

1x	____x	**Component**
32 µl		TE
10 µl		5X Terminal Transferase buffer
3 µl		25 mM CoCl2 (comes with TdT)
45 µl		

•Nucleotide Mix:

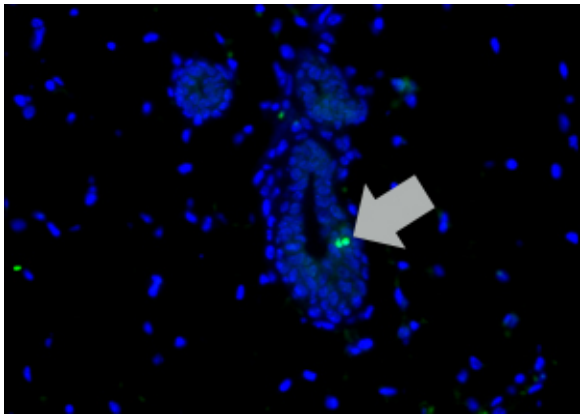
1x	____x	**Component**
0.25 µl		1 mM Alexa 488 dUTP
0.5 µl		1 mM dATP
4.25 µl		TE
5 µl		

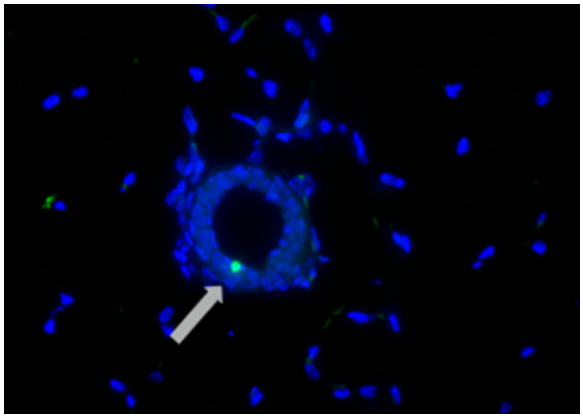
•Reaction Mix*:

1x	____x	**Component**
45 µl		Equilibration Buffer
5 µl		Nucleotide Mix
1 µl		TdT Enzyme [or TE]
51 µl		

*Make up one half with enzyme and the other without, replacing TdT with TE.

Representative Images of TUNEL staining:





• Original reference for TUNEL:

Gavrieli Y, Sherman Y, Ben-Sasson SA.
Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation.
J Cell Biol. 1992 Nov;119(3):493-501.

• Lab reference using our protocol:

Kingsley-Kallesen M, Mukhopadhyay SS, Wyszomierski SL, Schanler S, Schutz G, Rosen JM.
The mineralocorticoid receptor may compensate for the loss of the glucocorticoid receptor at specific stages of mammary gland development.
Mol Endocrinol. 2002 Sep;16(9):2008-18.