TACS® 2 TdT Replenisher and Core Reagent Kits

Catalog Numbers: 4810-30-R
4810-30-CK

INTENDED USE

TACS 2 TdT Replenisher and Core Reagent Kits provide only the reagents that are most rapidly exhausted from the complete TACS 2 TdT Apoptosis Detection Kits. The Replenisher Kit (R&D Systems, Catalog # 4810-30-R) and the Core Reagents Kit (R&D Systems, Catalog # 4810-30-CK) are also useful when using alternative detection and counterstaining methods in double-labeling experiments. Ensure familiarity with the complete "Assay Protocol" for the TACS 2 TdT kit of interest before relying on this abbreviated version.

MATERIALS PROVIDED & STORAGE CONDITIONS

Do not use past kit expiration date.

KIT*	PART	PART #	AMOUNT PROVIDED	STORAGE OF OPENED MATERIAL
R	**Proteinase K**	4800-30-01	50 µL	Store at ≤ -20 °C.
R, C	**TdT dNTP Mix**	4810-30-04	35 µL	
R, C	**TdT Enzyme**	4810-30-05	30 µL	
R, C	**50X Co^{2+}**	4810-30-09	30 µL	
R, C	**50X Mg^{2+}**	4810-30-10	30 µL	
R, C	**50X Mn^{2+}**	4810-30-14	50 µL	
C	**10X TdT Labeling Buffer**	4810-30-02	100 mL	Store at 2-8 °C.
C	**10X TdT Stop Buffer**	4810-30-03	100 mL	
R, C	**Strep-HRP**	4800-30-06	30 µL	

* R= Replenisher; C= Core Reagents

PRECAUTION

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

REFERENCES

1. Tomei, L.D. and F.O. Cope (1991) Current Comm. Cell. in Mol. Biol. **Vol 3.**
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5. Kerr, J.F. *et al.* (1995) Methods in Cell Biology **46**:1.
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REAGENT PREPARATION

Reagents marked with an asterisk (*) should be prepared immediately before use.

The volumes given for each reagent are based on processing samples of up to 4 cm^2 that are immobilized on glass slides. Different configurations of chamber slides, culture plates, free floating sections, and the use of glass coverslips may require adjustments to the stated volumes.

***Proteinase K Solution (included in Replenisher Kit only)** - Use 50 µL of Proteinase K Solution per sample. Store on ice. Thaw provided Proteinase K at room temperature, then place on ice. To prepare add:

Reaction Component	2 Samples	10 Samples	n Samples
Distilled water	100 µL	500 µL	n x 50 µL
Proteinase K	2.0 µL	10 µL	n x 1.0 µL

Under some circumstances, the Proteinase K may be used at a 1:200 dilution.

***Quenching Solution** - Use 50 mL of Quenching Solution to process 1-10 samples. To prepare add:

Reaction Component	Volume
30% hydrogen peroxide	5.0 mL
Methanol	45 mL

Always use fresh 30% hydrogen peroxide. It is recommended that 6 mL aliquots of fresh 30% hydrogen peroxide be made and stored at 2-8 °C. For each labeling procedure, use a fresh 30% hydrogen peroxide aliquot then discard the unused portion.

1X TdT Labeling Buffer (included in Core Reagent Kit only) - Dilute the 10X TdT Labeling Buffer 1:10 using distilled water. Leave at room temperature until use. Use 50 mL of 1X LdT Labeling Buffer to process 1-10 samples. Remove an aliquot of 50 µL per sample for preparing the Labeling Reaction Mix and place on ice.

***Labeling Reaction Mix -** Thaw TdT dNTP Mix at room temperature, then place on ice. To maintain optimal enzyme activity, remove the TdT Enzyme from freezer only long enough to pipette the required volume. Alternatively, place the TdT Enzyme in a ≤ -20 °C freezer block. Prepare the Labeling Reaction Mix just before use and keep the prepared reaction mix on ice. Prepare 50 µL per sample in the sequence given below:

Reaction Component	2 Samples	10 Samples	n Samples
TdT dNTP Mix	2.0 µL	10 µL	n x 1.0 µL
TdT Enzyme	2.0 µL	10 µL	n x 1.0 µL
50X Cation Stock**	2.0 µL	10 µL	n x 1.0 µL
1X TdT Labeling Buffer	100 µL	500 µL	n x 50 µL
**Select the proper cation by referring to the protocols in the appropriate TACS 2 kit.			

1X TdT Stop Buffer (included in Core Reagent Kit only) - Add the 10X TdT Stop Buffer 1:10 using distilled water. Leave at room temperature until use. Use 50 mL of 1X TdT Stop Buffer to process 1-10 samples.

***Strep-HRP Solution (if staining with DAB)** - Use 50 µL of Strep-HRP Solution per sample. Store prepared Strep-HRP Solution at room temperature until use. To prepare add:

Reaction Component	2 Samples	10 Samples	n Samples
1X PBS	100 µL	500 µL	n x 50 µL
Strep-HRP	2.0 µL	10 µL	n x 1.0 µL

***Strep-HRP Solution (if staining with TACS-Blue Label™)** - Use 50 µL of Strep-HRP Solution per sample. Store prepared Strep-HRP Solution at room temperature until use. To prepare add:

Reaction Component	Up to 15 Samples
Blue Strep-Diluent	500 µL
Strep-HRP	1.0 µL