Anti-hTERT Antibody (SCD-A7)

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Intended Use

This reagent is for in vitro diagnostic use.

Anti-hTERT Antibody (SCD-A7) is intended to be used for the qualitative identification by light microscopy of human telomerase reverse transcriptase (hTERT) protein in cytological specimens. The clinical interpretation of any staining or its absence should be complemented by cytological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Principle of Procedure

Anti-hTERT Antibody (SCD-A7) may be used as the primary antibody for immunocytochemical staining of cells. In general, immunocytochemical staining techniques allow for the visualisation of antigens via the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody that binds to the primary antibody, a tertiary antibody-enzyme complex, and a chromogenic substrate, with interposed washing steps. The enzymatic conversion of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and coverslipped. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

Summary and Explanation

Human telomerase reverse transcriptase (hTERT) is a 1132-amino acid protein that forms part of the ribonucleoprotein complex of human telomerase.[1,2] Telomerase is in part responsible for telomere maintenance, which is regarded as an important mechanism by which tumour cells evade senescence. In most cases this is achieved by reactivating or up-regulating telomerase activity.[3] In normal human cells, telomerase is strictly regulated during development.[4] Telomerase activity is extinguished during embryonic differentiation in most somatic cells, but remains active in highly proliferative tissues, such as germ cells, activated lymphocytes, and certain types of stem cell populations.[5] Telomerase is reported as activated in 80–90% of human carcinomas[6,7] and is present in circulating cancer cells.[8]

Clone

SCD-A7

Specificity

Human telomerase reverse transcriptase

Ig Class

IgM, kappa light chain

Reagent Provided

Anti-hTERT Antibody (SCD-A7) is a mouse monoclonal antibody produced as a tissue culture supernatant, and supplied in phosphate buffered saline with carrier protein, containing 0.05% ProClin®300 as a preservative.

Antibody Concentration

Between 5.4 and 6.6 µg/mL as determined by biolayer interferometry. Refer to vial label for lot-specific Ig concentration.

Reconstitution, Mixing, Dilution, Titration

Suggested dilution is 1/8–1/10. This is provided as a guide and users should determine their own optimal working dilutions. Antibody dilutions should be freshly prepared on the day of use.

Materials Required But Not Provided

1. PBS (phosphate buffered saline) pH 7.4
2. Refrigerated benchtop centrifuge
3. Thermo Scientific™ Shandon™ Cytospin™ Collection Fluid
4. Single cytology funnels with filter cards
5. Thermo Scientific Cytospin 4 Cytocentrifuge and Cytoclips
6. Sterile polypropylene centrifuge tubes, 50 mL
7. Microscope slides, positively charged
8. Mouse IgM kappa monoclonal isotype control
9. Positive and negative control cells
10. Ventana® BenchMark XT automated slide stainer
11. Ventana OptiView DAB IHC Detection Kit
12. Ventana Reaction Buffer Concentrate (10X)
13. Ventana Cell Conditioning Solution CC1
14. Ventana Antibody Dilution Buffer – to dilute primary antibody
15. Ventana DISCOVERY Ab Diluent – used as a blocking reagent
16. Ventana Hematoxylin II counterstain
17. Ventana Bluing Reagent
18. Deionised or distilled water
19. Ethanol or reagent alcohol
20. Xylene or xylene substitute (histological grade)
21. Mounting medium
22. Coverslips
23. Light microscope (40-400x)

Storage and Stability

Store the vial at 2-8°C (do not freeze) and return to storage conditions immediately after use. Storage conditions other than those recommended should be verified by the user.

Do not use the product beyond the expiration date shown on the antibody vial.

Warnings and Precautions

1. This product is for *in vitro* diagnostic use.
2. The concentration of ProClin 300 is 0.05%. It contains the active ingredients 2-methyl-4-isothiazolin-3-one and 5-chloro-2-methyl-4-isothiazolin-3-one. Wear appropriate personal protective equipment when handling this product, as exposure may cause irritation to the skin, eyes, mucous membranes and upper respiratory tract.
3. The concentration of ProClin 300 in this product does not meet the OSHA criteria for hazardous substance.
4. Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions.[9] Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents or specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amount of soap and water. Seek medical advice.
5. Avoid microbial contamination of reagents as this may produce incorrect results.
6. Consult local and/or state authorities to determine the recommended method of disposal.
7. Incubation times, temperatures and reagents, other than those specified, may give erroneous results. Any such changes must be validated by the user.
8. Further safety information is contained in the Safety Data Sheet, available on the website: **www.siennadiagnostics.com.au.**

Specimen Preparation

Anti-hTERT Antibody was validated using samples prepared with Shandon Cytospin Collection Fluid. Use of any other fixative requires prior validation.

This procedure is applicable to FNA (fine needle aspirate) cell suspensions, voided urine and other body fluid samples.

1. Samples collected fresh must be processed immediately, or maintained at 2-8°C for a period no longer than 48 hours prior to processing.
2. Centrifuge sample in 50 mL tubes at 470 g for 10 minutes at 2-8°C.
3. Discard the supernatant and resuspend the cell pellet in 10 mL PBS.
4. Centrifuge the samples at 470 g for 10 minutes at 2-8°C.
5. Discard the supernatant and inspect the cell pellet for large proteinaceous material. If present, repeat steps 3 and 4.
6. Resuspend the cell pellet in 10 mL Shandon Cytospin Collection Fluid
7. Centrifuge at 470 g for 10 minutes at 2-8°C.
8. Discard the supernatant and resuspend the cell pellet in Shandon Cytospin Collection Fluid (250 µL needed per slide).
9. Add 250 µL of the cell suspension to each cytology funnel attached via a cytoclip to a positively charged glass microscope slide.
10. Cytocentrifuge at 113g (1000 rpm) for 4 minutes with a low acceleration setting.
11. Separate the cytology funnel assemblies and continue with the staining procedure, or store slides at 2-8°C until use.

Staining Procedure

Anti-hTERT Antibody (SCD-A7) has been developed for use on a Ventana BenchMark XT automated slide stainer in combination with OptiView DAB IHC Detection Kit and accessories. Use of other staining procedures requires prior validation.

Recommended dilution for immunocytochemistry: 1/8–1/10

Recommended staining protocol:

Protocol Step	
Retrieval Conditions	CC1 - 8 mins @ 95°C
Pre-primary Peroxide Inhibitor	Selected
Antibody Incubation	36 mins @ 37°C
DISCOVERY Ab Diluent (Post Fixative)	16 mins
OptiView HQ Universal Linker	12 mins
OptiView HRP Multimer	12 mins
Counterstain	Hematoxylin II - 12 mins
Bluing Reagent	Bluing Reagent - 4 mins

Anti-hTERT Antibody (SCD-A7)

Quality Control

Differences in technical procedures in the user's laboratory may produce significant variability in results, necessitating regular performance of in-house controls in addition to the following procedures.

Positive Cell Control

The positive cell control is used to indicate correctly prepared cells and proper staining techniques. One positive cell control slide should be included for each set of test conditions in each staining run.

A cell with weak positive staining is more suitable than one with strong positive staining for optimal quality control and to detect minor levels of reagent degradation.[10]

If the positive cell control fails to demonstrate positive staining, results with the test specimens should be considered invalid.

Recommended positive control cells are peripheral blood mononuclear cells.

Negative Cell Control

The negative cell control should be examined after the positive cell control to verify the specificity of the labelling of the target antigen by the primary antibody. Non-specific staining, if present, usually has a diffuse appearance. If specific staining occurs in the negative cell control, results with the patient specimens should be considered invalid.

Recommended negative control cells are primary squamous epithelial cheek cells.

Negative Reagent Control

Use a non-specific negative reagent control in place of the primary antibody with each patient specimen to evaluate non-specific staining and allow better interpretation of specific staining at the antigen site.

Recommended negative reagent control is an isotype matched antibody of IgM, kappa light chain subclass at the same Ig concentration as the test (anti-hTERT) antibody.

False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes) or endogenous peroxidase (cytochrome C). To differentiate endogenous enzyme activity or non-specific binding of enzymes from specific immunoreactivity, additional patient cells may be stained exclusively with substrate chromogen or enzyme complexes (labelled polymer) and substrate chromogen, respectively.

Interpretation of Results

A positive result with Anti-hTERT Antibody (SCD-A7) is characterised by strong nuclear staining, with some cells also containing weak granular staining in the cytoplasm.

Limitations

1. Immunocytochemistry is a multiple step diagnostic process that requires specialised training in the selection of the appropriate reagents and cells, fixation, preparation of the immunocytochemistry slide, and the interpretation of the staining results.
2. Excessive or incomplete counterstaining may compromise proper interpretation of results.
3. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.
4. As with any immunocytochemistry test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells assayed.

Performance Characteristics

Anti-hTERT Antibody (SCD-A7) detected hTERT in normal and malignant cells in voided urine:

Cell Types	Normal Cases	Tumour Cases
Inflammatory cells: Lymphocytes, neutrophils, eosinophils	218/223	25/26
Normal urothelial cells	70/217	6/20
Atypical urothelial cells	36/56	11/11
Suspicious for high grade urothelial cells	7/9	3/3
High grade urothelial cells	2/2	3/3

- *Data is expressed as number of cases where hTERT is detected in a specific cell type / total number of cases in which the specific cell type was observed*
- *Cell type is determined by cytological evaluation based on morphology*
- *Normal and tumour cases are determined by cystoscopy/biopsy*

Anti-hTERT Antibody (SCD-A7) is recommended for the detection of hTERT protein in normal and neoplastic cytology samples.

References

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