

## ihcDirect® TTF1 Ab-Enh Anti-Human TTF1 (Clone R373)

Ab: K32041-015, 150 tissue stains\*  
 Ab: K32041-005, 50 tissue stains\*  
 Ab-Enh: K47041-015, 150 tissue stains\*  
 Ab-Enh: K47041-005, 50 tissue stains\*

### Intended Use: For *In Vitro* Diagnostic Use

Polymerized horseradish peroxidase (polyHRP)-labeled anti-TTF1 (R373) rabbit monoclonal antibody is intended for laboratory use to qualitatively identify by light microscopy the presence of TTF1 protein in sections of formalin-fixed, paraffin-embedded (FFPE) and/or fresh frozen tissues using immunohistochemistry (IHC) test methods. 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 and proper controls interpreted by a qualified pathologist and/or physician. This conjugated antibody has been pre-diluted and optimized for IHC use without further dilution.

### Summary and Explanation:

ihcDirect TTF1 is a polyHRP conjugated rabbit monoclonal antibody (clone R373). TTF1 is a transcription factor with a 38 kDa homeodomain region that is primarily expressed in lung and thyroid glands with critical functions in differentiation of these organs. It is also expressed in the CNS regions differentiated from the diencephalic region. TTF1 is commonly used to distinguish primary non-squamous lung carcinoma from metastatic carcinoma. Other applications of TTF1 include differentiating between lung adenocarcinoma and squamous cell carcinoma. Additionally, it distinguishes subependymal giant cell astrocytoma from gemistocytic astrocytoma and ganglioglioma which demonstrate similar histopathological features.

### Principle of Procedure:

The ready-to-use (RTU) ihcDirect TTF1 pHRP antibody conjugate is directly applied to pretreated tissue sections, where it binds to TTF1 antigens in tissue. Following antibody incubation, tissues are washed and a signal amplifier (ihc Enhancer) is applied. The tissues are washed again and a Working Solution (WS) of a chromogen such as ihc DAB 1:1 is applied to the tissue. The pHRP on the conjugate reacts with the chromogen to form a visible colored product at the site of TTF1 binding location. The specimen may then be counterstained and a coverslip applied. Results are viewed and interpreted using a light microscope. Volumes are based upon 100µl antibody per tissue. This product may be used to perform IHC manually or on an open automated IHC staining system.

### Reagents Provided:

Part No.	Σ	Description
K47041-015 K32041-015	150*	15ml size ihcDirect TTF1 RTU antibody conjugate and an equal volume of ihc Enhancer. <i>15ml size ihcDirect TTF1 RTU antibody conjugate.</i>
K47041-005 K32041-005	50*	5ml size ihcDirect TTF1 RTU antibody conjugate and an equal volume of ihc Enhancer. <i>5ml size ihcDirect TTF1 RTU antibody conjugate.</i>

\* At estimated volume of 100µl of antibody conjugate per tissue

Immunogen	Clone	Species	Ig Class	Total Protein Conc.
Recombinant TTF1	R373	Rabbit	IgG1	10 mg/ml

TTF1 antibody is a rabbit monoclonal antibody purified from ascites. HRP is extracted from horseradish plant. The ihc Enhancer is a signal amplification reagent. NovodiAx ihc DAB 1:1 Kit is recommended for use with the TTF1 antibody conjugate.

### TTF1 Ab-Enh Components (K47041-###):

Reagent Description	Component Part Numbers	Sizes (ml)
TTF1 pHRP	H32041-R### (005, 015)	5, 15
ihc Enhancer	D28020-R### (005, 015)	5, 15

### TTF1 Antibody Components (K32041-###):

Reagent Description	Component Part Numbers	Sizes (ml)
TTF1 pHRP	H32041-R### (005, 015)	5, 15

### Ancillary Reagents for Use with TTF1 pHRP Antibody:

Reagent Description	Part Numbers	Sizes (ml)
ihc Blocker	Intl. K51001-### (015) USA K51002-### (015)	15 15
ihc Enhancer	K51011-### (015)	15
ihc DAB 1:1 Kit	K50002-### (015, 030)	15, 30
ihc Magenta 1:1 Kit	K50011-### (015, 030)	15, 30

### Materials Needed but Not Provided:

The following reagents/supplies may be required in staining but are not provided:

- |   |  |
|---|--|
| 1. Frozen section fixative (Acetone and/or 10% NBF§)  | 9. Peroxide blocker (optional)   |
| 2. Positive and negative control tissues              | 10. Instruments used for tissue pretreatment, such as water bath, or pressure cooker or microwave oven (when using FFPE tissues) |
| 3. Microscope slides, positively charged (required)   | 11. Hematoxylin  |
| 4. Staining jars, baths or processing tools           | 12. Xylene or Xylene substitute  |
| 5. ihc Wash Buffer (PBS-T)                            | 13. Ethanol  |
| 6. Pipettor and pipet tips                            | 14. Mounting medium  |
| 7. Timer  | 15. Cover slips  |
| 8. Antigen retrieval buffer (when using FFPE tissues) | 16. Light microscope (40-420x)   |
- § NBF – neutral buffered formalin

### NovodiAx Bulk Reagent Formulations:

- 1X ihc Wash Buffer (PBS-T), (10 mM phosphate buffer, pH7.2, 150 mM NaCl, 0.05% Tween-20).
- Antigen Retrieval Buffer (10mM Citric buffer, pH 6.0, 0.05% Tween 20).

### Storage and Handling:

This product should be stored at 2-8°C and is suitable for use until expiration date when stored at this temperature. Do not freeze. Do not use the product after expiration date unless dating extension information is provided by NovodiAx. If reagents are stored under any conditions other than those specified in the package insert, they must be validated by the user.

### Specimen Preparation:

**Paraffin Sections:** Tissues routinely processed with 10% NBF are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981; Sheehan & Hrapchak, 1980). Variable results may occur as a result of prolonged fixation. Each section should be cut to the appropriate thickness (approximately 4-5 µm) and placed on a positively charged glass slide. Slides containing the tissue section may be baked for at least one hour but not exceeding 24 hours in a 58-60°C±5°C oven. Osseous tissues should be decalcified prior to tissue processing to facilitate tissue cutting and prevent damage to microtome blades (Kiernan, 1981; Sheehan & Hrapchak, 1980).

**Frozen Tissue Sections:** Frozen tissue is sectioned to the appropriate thickness (approximately 5 µm) and placed on a positively charged glass slide. Tissues



should be fixed in reagent grade 10% NBF for 1-2 minutes immediately after sectioning. Reagent grade NBF may be kept cold, e.g. cryostat temperatures, or room temperature. Following fixation, tissues should be processed within a few minutes or may be stored in PBS for several hours.

**Treatment of Tissues Prior to Staining:** Pretreatment is tissue dependent and should be performed as suggested in the staining procedure sections.

**Warnings and Precautions:**

1. Read and understand all of the Novodiox Instructions for Use (IFUs) before product use.
2. The ihcDirect TTF1 antibody conjugate and ihc Enhancer signal amplification reagent are pre-diluted. Further dilution may reduce signal intensity or increase the possibility of false-negative staining. These recommendations are for guidance only. Laboratory managers should determine their own procedures and quality policies.
3. To obtain best results when working with frozen tissues, it is desirable to freeze tissues as quickly as possible following extraction.
4. Use caution and shorten incubation times when utilizing intense hematoxylin counterstains such as Gills as these stains may tend to mask antibody staining.
5. Take reasonable precautions when handling reagents. Use protective equipment such as disposable gloves and lab coats when handling materials. Read Safety Data Sheets (SDS) prior to use.
6. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
7. Use charged slides to secure tissue adhesion.
8. Patient specimens and all materials that come into contact with patient specimens should be handled as bio-hazardous materials and disposed of appropriately.
9. Consult local or state authorities with regard to recommended method of disposal of bio-hazardous and hazardous chemical waste materials.
10. Incubation time and temperature other than those specified may give erroneous results. The user must validate any such changes.
11. Use lab grade quality chemicals such as NBF and water when preparing reagents. Users should validate performance including stability for laboratory prepared reagents (at 1x).
12. Avoid microbial contamination of reagents.
13. Fixation is a vital part of the protocol and fixation times may vary with the fixative chosen, tissue type, e.g. containing fat and other parameters. Generally, an acetone or NBF fixation of 1-2 minutes is recommended. Place frozen tissue sections into fixation solution shortly after sectioning. **Prolonged exposure to room or freezing temperatures may alter targeted epitopes.**
14. It is best to prevent slides from drying out during the staining process to avoid unwanted background staining

**Staining Procedures:**

General Operating Notes:TTF1

1. Equilibrate all reagents to room temperature prior to use. Swirl or shake the ihc Enhancer and pHRP-labeled antibody solutions before use. **Do not vortex.** Calculate the amount of chromogen WS needed (100µl per tissue) and **freshly** prepare chromogen WS. See instructions for use.
2. Gently and thoroughly wash tissues during manual wash steps. Avoid direct high velocity streams of wash that might tend to damage or cut delicate tissues.
3. Following each manual assay step, remove excess fluids on tissue slides with tissue paper. Excessive residual solution may dilute subsequent reagents, causing negative or uneven staining. Users may also utilize a PAP pen to ensure reagents stay on the desired tissues.
4. To reduce background signal, wash thoroughly following antibody and enhancer step.
5. For the tissues with high oxidase activity, blocking with H<sub>2</sub>O<sub>2</sub> may be required to minimize non-specific staining.
6. The following protocol has been validated at temperatures between 21°-30°C (71.6°-86°F) for incubating TTF1 pHRP and ihc Enhancer and a Novodiox chromogen WS. If room temperature is less than 21°C, users may need to incubate labeled antibody for a longer period of time (<5 minutes) to achieve satisfactory staining results. Consistent results have been obtained at room temperature or using a slide warmer set to 30°C at the surface of the slide.

**Frozen Tissue Sections:**

1. Following fixation in NBF, rinse slides with 1x ihc Wash buffer and then wipe away any excess fluid with a Kimwipe® or paper towel.

2. Dispense 100µl of pHRP antibody, covering the entire tissue, and incubate for 3 minutes at room temperature. If temperatures are below 21°C manual users may extend incubation times. Then, thoroughly rinse slides with 1x ihc Wash buffer and wipe away any excess fluid.
3. Dispense 100µl of ihc Enhancer, covering the entire tissue, and incubate at room temperature for 3 minutes. Then, thoroughly rinse slides with 1x ihc Wash buffer and wipe away any excess fluid.
4. Dispense 100µl of a chromogen WS, such as DAB covering the entire tissue, and incubate for 1-3 minutes at room temperature. Users should determine the optimal incubation time for their particular chromogen or lab environment. Then rinse slides with either 1x ihc Wash buffer or lab grade water and wipe away any excess fluid.
5. Add a counterstain. Incubation times will vary according to the counterstain formulation. Then, rinse slides with water and wipe away any excess fluid.
6. Apply aqueous media or dehydrate slides with the user's typical dehydration protocol and permanent mounting, then add coverslip.

**Test Timing Est. (10-12 minute IHC protocol for frozen tissue sections):**

ihcDirect Antibody+Enhancer Frozen Tissue Procedure	Time in minutes
Fix with Acetone or Neutral Buffered Formalin	Start
- Wash with ihc Wash Buffer, remove excess fluid	- - -
<i>*Optional: Block with ihc Blocker</i>	1
- Tap+Absorb to remove excess blocker	- - -
Novodiox ihcDirect pHRP Antibody	3
- Wash thoroughly with ihc Wash Buffer	- - -
- Tap+Absorb to remove excess wash buffer	- - -
ihc Enhancer	3
- Wash thoroughly with ihc Wash Buffer	- - -
- Tap+Absorb to remove excess wash buffer	- - -
Novodiox Chromogen Working Solution	1-3
- Wash with ihc Wash Buffer or DI water	- - -
Hematoxylin counterstain (Conc. Dependent)	2-45 sec
Wash with water	- - -
Dehydrate/Mounting Medium and Coverslip	User Det.
<b>Total</b>	<b>~10-12</b>

**Paraffin Tissues:**

1. Deparaffinization: Soak slides in Xylene 3 times for 5 minutes each. Next, 3 minutes each in 100%, 95% and 75% ethanol. Then wash slides with tap water in slide tank for two times, 2 minutes each time.
2. Antigen retrieval: Using a water bath, incubate slides in antigen retrieval buffer in a slide tank at 96°C for 30 minutes, then cool the slides down to room temperature for 30 minutes. Rinse the slides twice with tap water, 2 minutes each time.
3. (Optional) Block tissue with H<sub>2</sub>O<sub>2</sub>: Soak the slides in 3% H<sub>2</sub>O<sub>2</sub> in slide tank, stand for 10 minutes. Rinse the slides with tap water twice and then wash once with PBS-T in slide tank for 2 minutes. Remove excess fluid.
4. Dispense 100µl of pHRP labeled anti-human TTF1 antibody on slides covering the entire tissue and incubate for 30 minutes at room temperature. Rinse the slides three times with PBS-T in slide tank, 2 minutes each time. Remove excess fluid.  
Note: Place slides in a wet chamber during antibody incubation step to prevent evaporation when longer incubation times are used.
5. Dispense 100µl of a chromogen WS covering the entire tissue, incubate for 3 minutes at room temperature. Rinse the slides twice with DI or tap water in slide tank, 2 minutes each time.
6. Counterstaining: Add hematoxylin and incubate for 1 minute at room temperature. Rinse twice with tap water for 2 minutes, each time.
7. Dehydration: Soak slides in the following order: 75% ethanol for 3 minutes, 95% ethanol for 3 minutes, 100% ethanol for 3 minutes and Xylene twice at 5 minutes each time.



- Applying Coverslip: Add one drop of permanent mounting medium on both the slide and the coverslip, then apply coverslip.

#### Quality Control Procedures:

Positive and negative controls should be run simultaneously with patient specimens.

**Positive Tissue Control:** The recommended positive control tissues for this antibody are established TTF1 antibody positive tissues. One positive tissue control for each set of test conditions should be included in each staining run. Previously frozen, freshly cut tissues and in some cases, an individual's own tissue may be used as controls.

Tissues used for positive controls should be selected from patient specimens with characterized positive target activity that give positive staining results. Established positive tissue controls should only be utilized for monitoring correct performance of processed tissues and test reagents, rather than in formulating a specific diagnosis of patient samples. If positive tissue controls fail to demonstrate positive staining, patient specimen results should be considered invalid.

**Negative Tissue Control:** The same tissue may be used to provide positive and negative controls. Differentiation of cells present in most tissue sections provide internal negative control sites, but this should be verified by the user. Components that do not stain should lack antibody specific staining, and provide an indication of non-specific background staining. If specific staining (false positive staining) occurs in the negative tissue control sites, results with the patient specimens must be considered invalid.

#### Troubleshooting:

If unexpected staining occurs on control tissues or patient samples, consider the following:

- No staining:** If no staining is evident on positive control slide, please verify whether (1) chromogen WS was prepared freshly and correctly, (2) reagents were applied in the correct order, (3) pHRP-labeled antibody was indeed added, and (4) for FFPE tissue, dewaxing and antigen retrieval were performed adequately. Perform any corrective actions required and then repeat the procedure.
- Low signal or faint staining:** Please check whether (1) the reagents are not expired, (2) temperature of the testing environment was at least 21°C or a 30°C slide warmer was used, (3) chromogen WS was prepared freshly and correctly, (4) excess ihc Wash solution was not left on the slide, causing subsequent reagents to be diluted, and (5) for FFPE tissue, dewaxing and antigen retrieval were performed adequately. Perform any required corrective actions and repeat the procedure. Alternatively, if using a DAB chromogen, consider using another stain, e.g. ihc Magenta 1:1 to obtain more vibrant staining. In addition, some individuals may naturally have low expression of certain antigens. In these cases, users may extend the incDirect antibody incubation times by 1-2 minutes.
- High background:** Possible causes include (1) insufficient washing, (2) specimen drying out, (3) prolonged chromogen incubation, (4) prolonged pHRP-labeled antibody incubation, and (5) specimen containing high level of endogenous peroxidase, which necessitates an additional blocking step (refer to the Staining Procedures for Paraffin Tissues). Perform any required corrective actions and repeat the procedure.

If unexpected staining is observed on control tissues or patient samples which cannot be explained by variations in laboratory procedures or a problem with the antibody is suspected, contact NovodiAx Technical Support or your local distributor immediately. Within the US and Canada call 1 (888) 439-2716 ext. 2 or 1 (510) 342-3043 ext. 2.

#### Expected Results:

Intense color stains the tissue with a clean background if TTF1-expressed cells exist. There will be no color staining if no TTF1-expression cells exist in the tissue. Interpretation of the staining result is solely the responsibility of the user.

#### General Limitations:


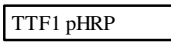

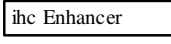








Immunohistochemistry is a multistep diagnostic process that requires specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results. Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation

and embedding methods, or to inherent irregularities within the tissue (Nadji M, Morales AR. 1983).

The manufacturer provides these antibodies/reagents at optimal dilution for use following the provided instructions for IHC on prepared tissue sections. Any deviation from recommended test procedures may invalidate declared expected results; appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results under these circumstances.

#### Performance Characteristics:

The ihcDirect TTF1 pHRP test performance has been determined using both frozen and FFPE tissue sections. NovodiAx has conducted studies to evaluate the performance of the antibody conjugates, accompanying reagents and ancillary supplies. The antibodies and systems have been found to be sensitive and demonstrate high specificity in binding to the antigen of interest. NovodiAx antibodies and accompanying reagents have demonstrated reliable and consistent results between runs and across lot numbers. These products have been determined to be stable for the periods of time specified on the labels either by standard real-time and/or accelerated methods. NovodiAx ensures product quality by testing each lot of material by testing materials at regular intervals and via surveillance programs.

Key to Symbols			
	<i>In vitro</i> diagnostic medical device		pHRP TTF1 antibody conjugate
	Catalog Number		pHRP Enhancing amplification reagent
	Use by: YYYY-MM-DD		Manufacturer
	Consult Instruction for Use		Contains sufficient for < n > tests
	Batch Code		CE Mark
	Temperature Limitations		Authorized European Representative

#### Instructions for Use (IFU) Access:

To obtain a translation or the latest electronic version of an IFU document, visit our website at <https://www.novodiAx.com/literature/instructions-for-use-ifu/>. Printed copies of an IFU document may be obtained by contacting NovodiAx Technical Support or your local distributor.

#### Bibliography:

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- Misch D., et al. Value of thyroid transcription factor (TTF)-I for diagnosis and prognosis of patients with locally advanced or metastatic small cell lung cancer. Diagn Pathol. 2015; 10:2 1 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4391673/>.
- Cardnell R., et al Protein expression of TTF1 and cMYC define distinct molecular subgroups of small cell lung cancer with unique vulnerabilities to aurora kinase inhibition, DLL3 targeting, and other targeted therapies. Oncotarget.8 201 7: 73419-73432. <https://www.oncotarget.com/article/20621/text/>.

