# abcam

## Product datasheet

## Anti-5T4 antibody [EPR5530] - BSA and Azide free ab189508



Recombinant

RabMAb

## 9 Images

#### Overview

**Product name** Anti-5T4 antibody [EPR5530] - BSA and Azide free

**Description** Rabbit monoclonal [EPR5530] to 5T4 - BSA and Azide free

**Host species** Rabbit

Suitable for: IP, WB **Tested applications** 

Unsuitable for: Flow Cyt,ICC/IF or IHC-P

Species reactivity Reacts with: Rat, Human

Predicted to work with: Mouse

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control HEK-293T, HT 1376, HeLa, JAR and MCF7 cell lysates; MCF7 cells.

**General notes** ab189508 is the carrier-free version of ab129058.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

#### **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR5530

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab189508 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 72-80 kDa (predicted molecular weight: 46 kDa).

**Application notes** Is unsuitable for Flow Cyt,ICC/IF or IHC-P.

#### **Target**

**Tissue specificity** Expressed by all types of trophoblasts as early as 9 weeks of development. Specific for

trophoblastic cells except for amniotic epithelium. In adult tissues, the expression is limited to a

few epithelial cell types but is found on a variety of carcinoma.

**Sequence similarities** Contains 6 LRR (leucine-rich) repeats.

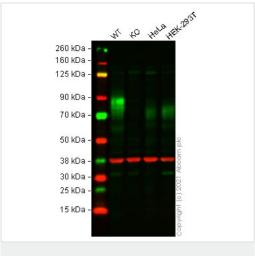
Contains 1 LRRCT domain. Contains 1 LRRNT domain.

**Cellular localization** Membrane.

Form 5T4 is a 72 kDa transmembrane glycoprotein that exhibits restricted expression in human and

mouse adult tissues, is upregulated on many carcinomas and tumour expression correlates with poorer clinical outcome in ovarian, gastric and colorectal cancers. mES cells lack cell surface expression of the 5T4 antigen, and both protein and mRNA are rapidly upregulated following induction of differentiation. This proteins expression correlates with downregulation of OCT-4 and

Tra-1-60 (Ward et al., 2006).



Western blot - Anti-5T4 antibody [EPR5530] - BSA and Azide free (ab189508)

**All lanes :** Anti-5T4 antibody [EPR5530] (ab129058) at 1/1000 dilution

**Lane 1 :** Wild-type MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lane 2: TPBG knockout MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

**Lane 3 :** HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 4 :** HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lysates/proteins at 20 µg per lane.

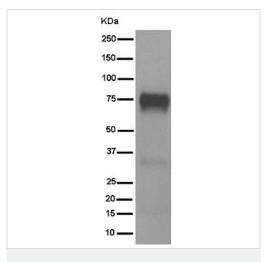
Performed under reducing conditions.

Predicted band size: 46 kDa
Observed band size: 85 kDa

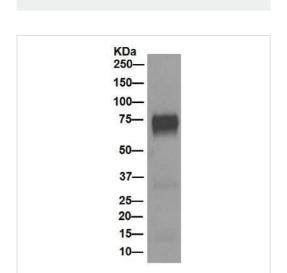
This data was developed using the same antibody clone in a different buffer formulation (<u>ab129058</u>).

**Lanes 1 - 4:** Merged signal (red and green). Green - <u>ab129058</u> observed at 85 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab129058 was shown to react with 5T4 in wild-type MCF7 cells in Western blot with loss of signal observed in TPBG knockout cell line ab269499 (knockout cell lysate ab269661). Wild-type MCF7 and TPBG knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in before incubation with ab129058 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-5T4 antibody [EPR5530] - BSA and Azide free (ab189508)



Western blot - Anti-5T4 antibody [EPR5530] - BSA and Azide free (ab189508)

Anti-5T4 antibody [EPR5530] ( $\underline{ab129058}$ ) at 1/5000 dilution (purified) + Rat brain at 20  $\mu g$ 

## Secondary

HRP goat-anti rabbit (H+L) at 1/1000 dilution

Predicted band size: 46 kDa

This data was developed using <u>ab129058</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

Anti-5T4 antibody [EPR5530] ( $\underline{ab129058}$ ) at 1/2000 dilution (unpurified) + Rat brain at 20  $\mu g$ 

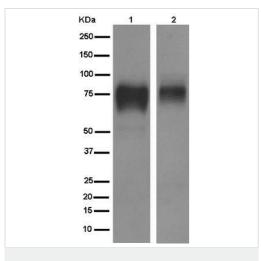
#### Secondary

HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 46 kDa

This data was developed using <u>ab129058</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-5T4 antibody [EPR5530] - BSA and Azide free (ab189508)

**All lanes :** Anti-5T4 antibody [EPR5530] (<u>ab129058</u>) at 1/5000 dilution (purified)

Lane 1 : HT-1376 cell lysate
Lane 2 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

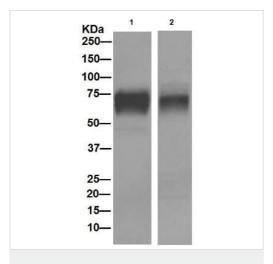
**Secondary** 

All lanes: HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 46 kDa

This data was developed using <u>ab129058</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-5T4 antibody [EPR5530] - BSA and Azide free (ab189508)

**All lanes :** Anti-5T4 antibody [EPR5530] (**ab129058**) at 1/2000 dilution (unpurified)

Lane 1 : HT-1376 cell lysate

Lane 2 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

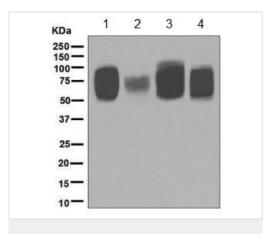
**Secondary** 

All lanes: HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 46 kDa

This data was developed using <u>ab129058</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-5T4 antibody [EPR5530] - BSA and Azide free (ab189508)

**All lanes :** Anti-5T4 antibody [EPR5530] (<u>ab129058</u>) at 1/1000 dilution (unpurified)

Lane 1: HT 1376 cell lysate
Lane 2: HeLa cell lysate
Lane 3: JAR cell lysate
Lane 4: MCF-7 cell lysate

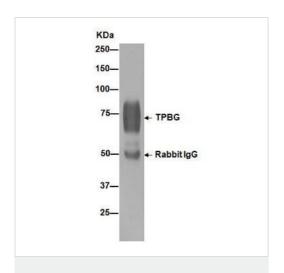
Lysates/proteins at 10 µg per lane.

## Secondary

All lanes: Goat anti-rabbit HRP conjugated at 1/2000 dilution

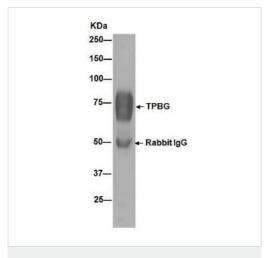
Predicted band size: 46 kDa

This data was developed using <u>ab129058</u>, the same antibody clone in a different buffer formulation.



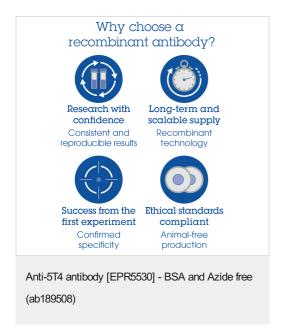
Immunoprecipitation - Anti-5T4 antibody [EPR5530] - BSA and Azide free (ab189508)

This data was developed using <u>ab129058</u>, the same antibody clone in a different buffer formulation.<u>ab129058</u> (unpurified) at 1/50 immunoprecipitating 5T4 in MCF-7 (Lane 1). For western blotting, a HRP goat anti-rabbit was used as the secondary antibody (1/1000).Blocking buffer and concentration: 5% NFDM/TBST.Diluting buffer and concentration: 5% NFDM/TBST.



This data was developed using <u>ab129058</u>, the same antibody clone in a different buffer formulation.<u>ab129058</u> (unpurified) at 1/50 immunoprecipitating 5T4 in MCF-7 (Lane 1). For western blotting, a HRP goat anti-rabbit was used as the secondary antibody (1/1000).Blocking buffer and concentration: 5% NFDM/TBST.Diluting buffer and concentration: 5% NFDM/TBST.

Immunoprecipitation - Anti-5T4 antibody [EPR5530] - BSA and Azide free (ab189508)



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