Validation Report #029645

Validation Date: 03/15/14

Summary

Antigen	Mitogen-Activated Protein Kinase Kinase 5 (MAP2K5)
Catalog number	ABIN754183
Supplier	Bioss
Supplier catalog number	bs-4124r
Lot number	120828
Method validated	Immunohistochemistry
Laboratory	Immunohistochemistry Core, NYU Langone
Validation number	029644
Positive Control	Human breast myoepithelial and glandular tissue
Negative Control	Human breast adipose tissue
Notes	Strong signal was detected in positive control sample, with minor background seen in negative control sample.

Full Methods

Primary Antibody
- Antigen: Mitogen-Activated Protein Kinase Kinase 5 (MAP2K5)
- Catalog number: ABIN754183
- Supplier: Bioss
- Supplier catalog number: bs-4124r
- Lot number: 120828

Isotype Control Antibody
- Antibody: Rabbit IgG isotype control
- Supplier: Ventana Medical Systems
- Catalog number: 790-2014
- Lot number: C11245

Secondary Antibody
- Antibody: Biotinylated goat anti-rabbit/anti-mouse (Kit)
- Supplier: Ventana Medical Systems
- Catalog number: 760-091
- Lot number: D07640BA

Additional Information

Detection kit information:
- Type: iView Streptavidin Peroxidase DAB
- Supplier: Ventana Medical Systems
- Catalog number: 760-091
- Lot number: D07640A

Controls
- Positive control: Human breast myoepithelial and glandular tissue stained with antibody
- Negative control: Human breast adipose tissue stained with antibody
- Isotype control: Human breast myoepithelial and glandular tissue stained with isotype control
- Secondary only control: Human breast myoepithelial and glandular tissue stained with secondary antibody only

Protocol

Immunohistochemistry was performed on a Ventana NEXes automated platform; instrument manufacturer specific reagents are italicized.

1. Slides were preheated in convection oven at 60°C for 30 min
2. Deparaffinization procedure:
    - 3 changes of Xylene, 5 min each
    - 3 changes of 100% Ethanol, 3 min each
    - 3 changes of 95% Ethanol, 3 min each
    - Rinsed in distilled water, 3 changes
3. Heat retrieval procedure
    - Slides retrieved in 10.0 mM Citrate, pH6.0 in a 1000W microwave oven (~100°C) for 15 min.
    - Slides were allowed to cool (in citrate) for 30 min.
    - Slides were washed x 3 in Distilled water
4. NEXes instrument procedure, iView DAB paraffin protocol (*abridged*):
    - Slide chamber warmed to 37°C
5. Slides rinsed with *reaction buffer* x3
6. *iView Inhibitor (H2O2)* applied and incubated for 4 min
7. Slides rinsed with *reaction buffer*
8. Antibody Application
    - Primary antibody diluted 1:250 in PBS (100 microliter applied/slide)
    - Ventana Isotype control applied neat
    - Slides Incubated overnight at room temperature (~12 hours ~25°C)

9. Slides rinsed with *reaction buffer* x3
10. *iView Biotinylated IgG* applied and incubated for 8 min
11. Slides rinsed with *reaction buffer*
12. *iView Streptavidin-Horseradish Peroxidase* applied and incubated for 8 min
13. Slides rinsed with *reaction buffer*
14. *iView DAB/H2O2* applied and incubated for 8 min
15. Slides rinsed with *reaction buffer*
16. *iView Copper* applied and incubated for 4 min
17. Slides rinsed with *reaction buffer*
18. Slides washed in Dawn Detergent/tap water
19. Counterstain Procedure
    - Hematoxylin (Leica 560 MX) 30 sec
    - Slides washed in tap water, 1 min
    - Decolorized (10% Acetic Acid in 70% ethanol), 1 min
    - Slides washed in tap water, 1 min
    - Bluing (Austin Clear Ammonia), 1 min
    - Slides washed in tap water, 1 min

20. Dehydration/coverslipping procedure:
    - 3 changes of 95% Ethanol, 3 min each
    - 3 changes of 100% Ethanol, 3 min each
    - 3 changes of Xylene, 5 min each
    - Mounted with Permount

21. Imaging: Leica SCN 400F Whole Slide Scanner with Digital Image Hub and Leica Slidepath software

Experimental Notes

Deviations from protocol/procedure supplied by manufacturer (attached).

- Step 1: Heated tissue 60°C for 30 minutes; manufacturer heats for 45 minutes.
- Step 2: No ethanol wash was performed during deparaffinization; manufacturer includes 1 wash of 80% ethanol for 3 minutes.
- Step 3.1: Slides were heated for 15 minutes; manufacturer provides a range of 15-20 minutes.
- Step 3.2: Slides were cooled for 30 minutes; manufacturer cools for 20 minutes.
- Step 4: Italicized reagents and incubation time are fixed instrument parameters.
- Step 5: Secondary species-specific serum block not used; manufacturer blocks with 5% normal goat serum for 2 hours.
- Step 8.1: Antibody diluted in PBS at 1:250; manufacture did not recommend diluent or dilution.
- Step 8.2.1: Primary antibody incubated at room temperature overnight; manufacturer incubates overnight 4°C with agitation.

Figures

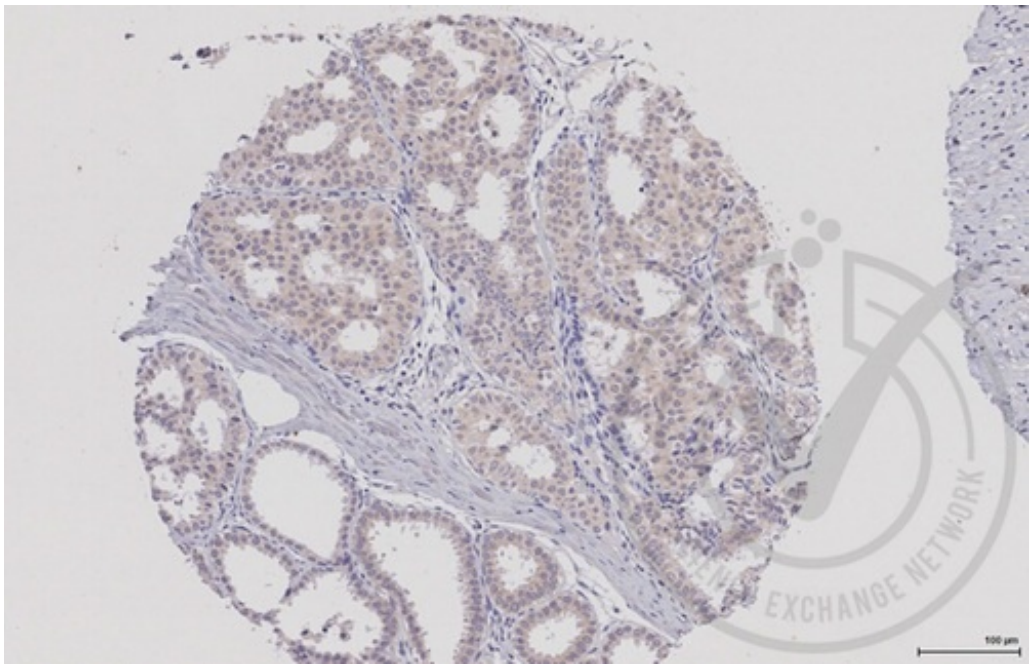

Figure 1: Human breast tissue stained with anti-MAP2K5 (brown) and counterstained with hematoxylin.

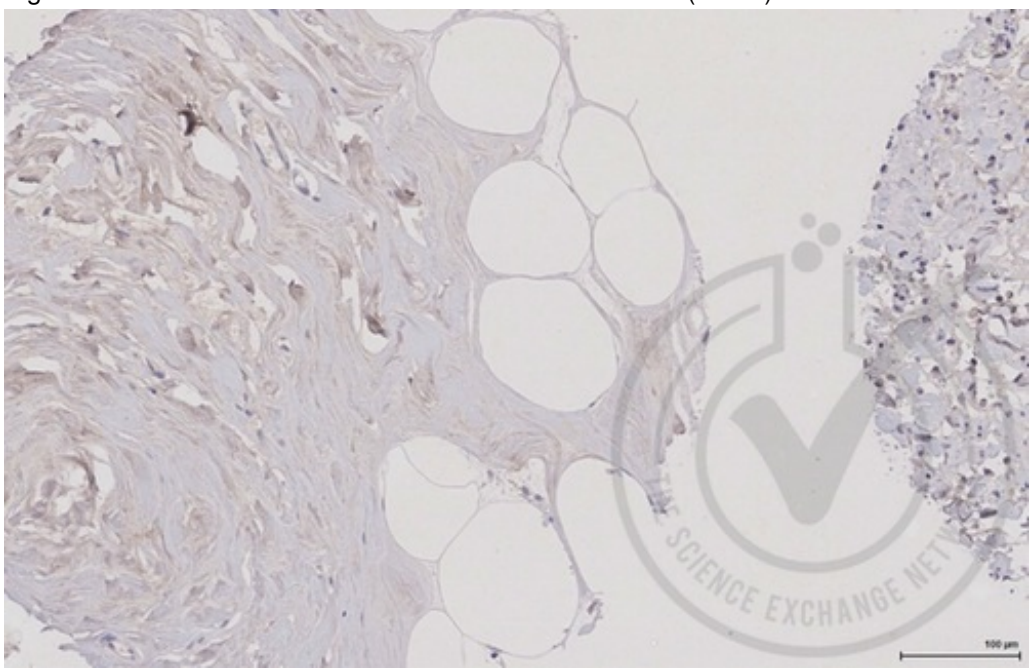

Figure 2: Human breast adipose tissue stained with anti-MAP2K5 (brown) and counterstained with hematoxylin.

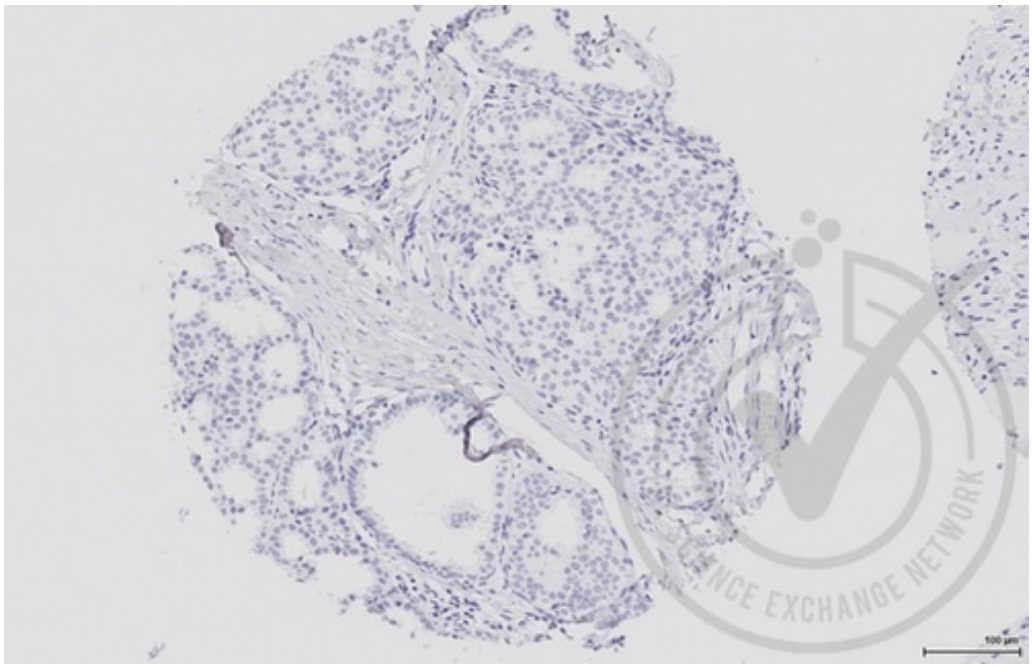

Figure 3: Human breast tissue stained with isotype control antibody (brown) and counterstained with hematoxylin.

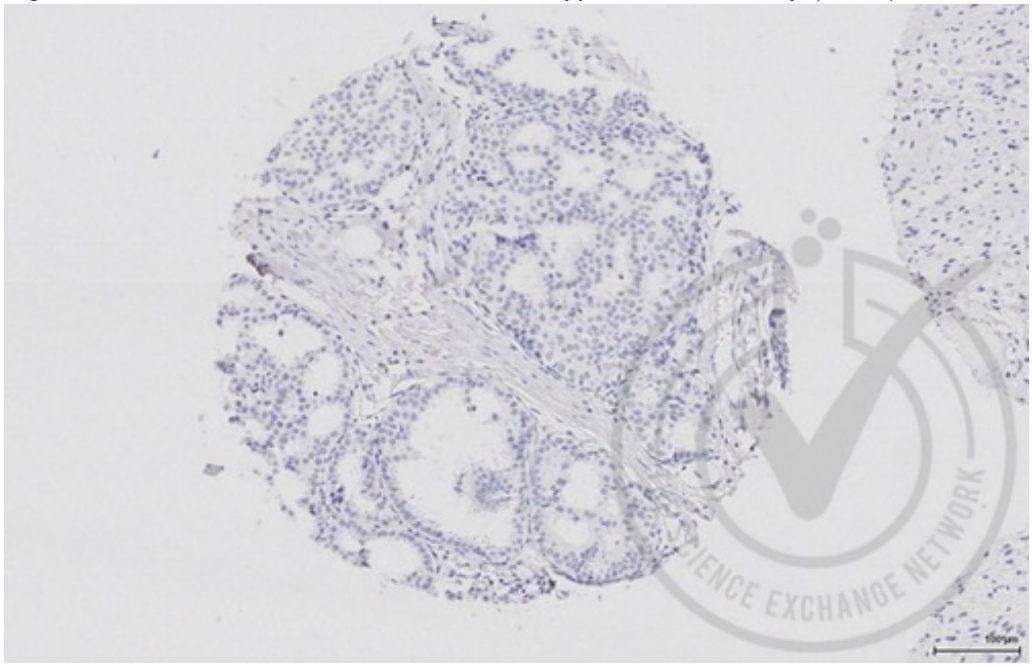

Figure 4: Human breast tissue stained with secondary antibody only (brown) and counterstained with hematoxylin.