

Validation Report #029662

Validation Date: 04/09/14

Summary

Antigen	Mitogen-Activated Protein Kinase 14 (MAPK14) (pThr180), (pTyr182)
Catalog number	ABIN678668
Supplier	Bioss
Supplier catalog number	bs-2210R
Lot number	140106
Method validated	Immunohistochemistry
Laboratory	Immunohistochemistry Core, NYU Langone
Validation number	029662
Positive Control	Human placenta tissue
Negative Control	Human tonsil tissue (no literature evidence of phospho-p38 MAPK in tonsil)
Notes	Signal is detected in positive control tissue and not in negative control tissue.



Full Methods

Primary Antibody

- Antigen: Mitogen-Activated Protein Kinase 14 (MAPK14) (pThr180), (pTyr182)
- Catalog number: ABIN678668
- Supplier: Bioss
- Supplier catalog number: bs-2210R
- Lot number: 140106

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 placenta tissue stained with antibody
- Negative control: Human tonsil tissue stained with antibody
- Isotype control: Human placenta tissue stained with isotype control
- Secondary only control: Human placenta 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/cover slipping 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:

- 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

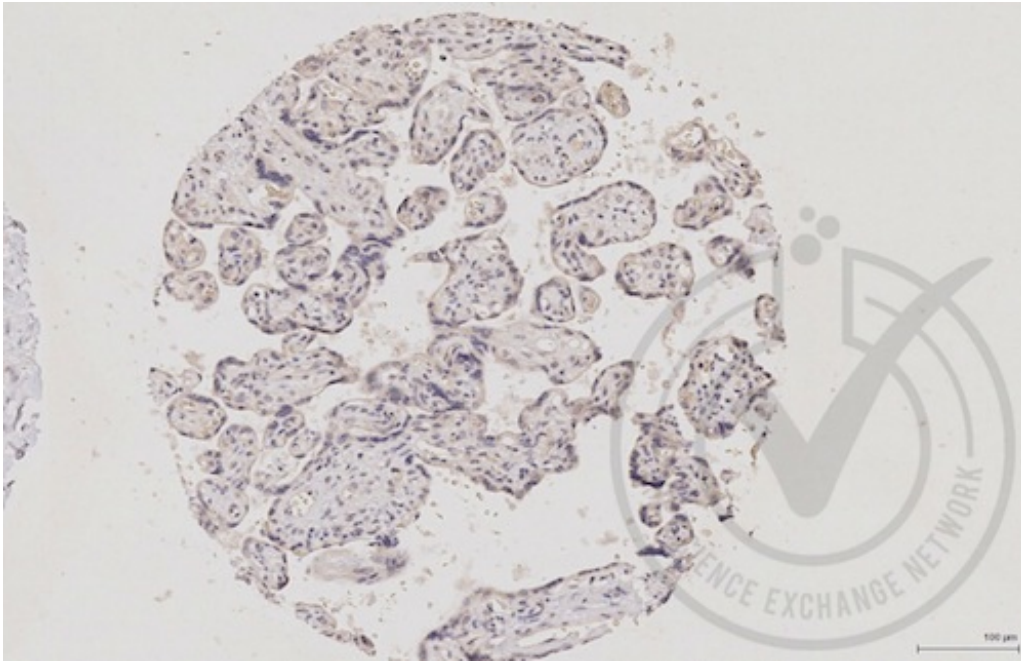


Figure 1: Human placenta tissue stained with anti-phospho-MAPK14 (brown) and counterstained with hematoxylin.

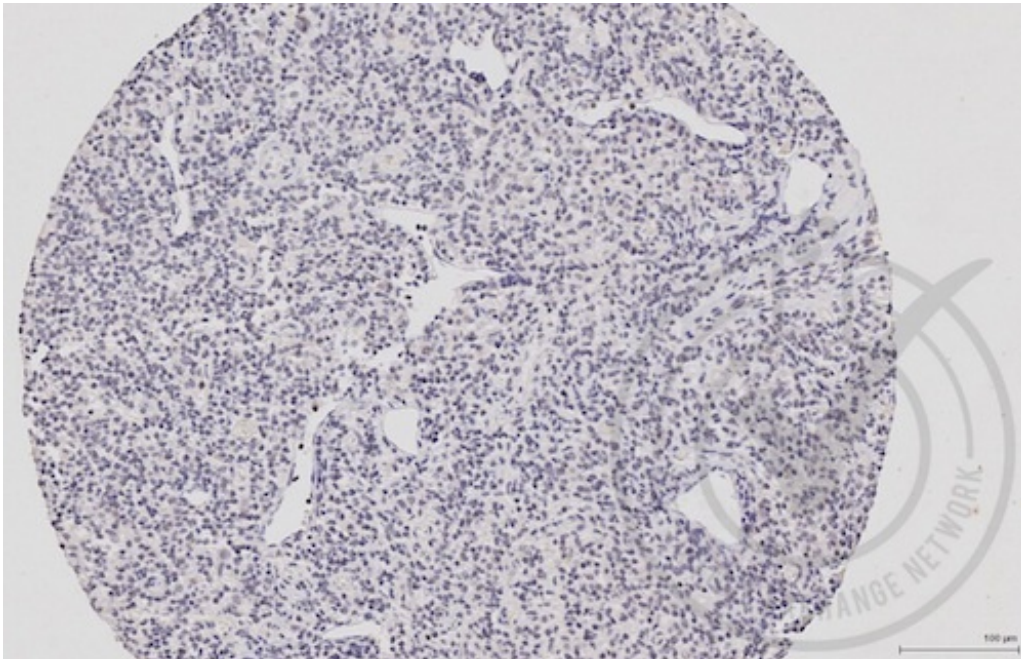


Figure 2: Human tonsil tissue stained with anti-phospho-MAPK14 (brown) and counterstained with hematoxylin.

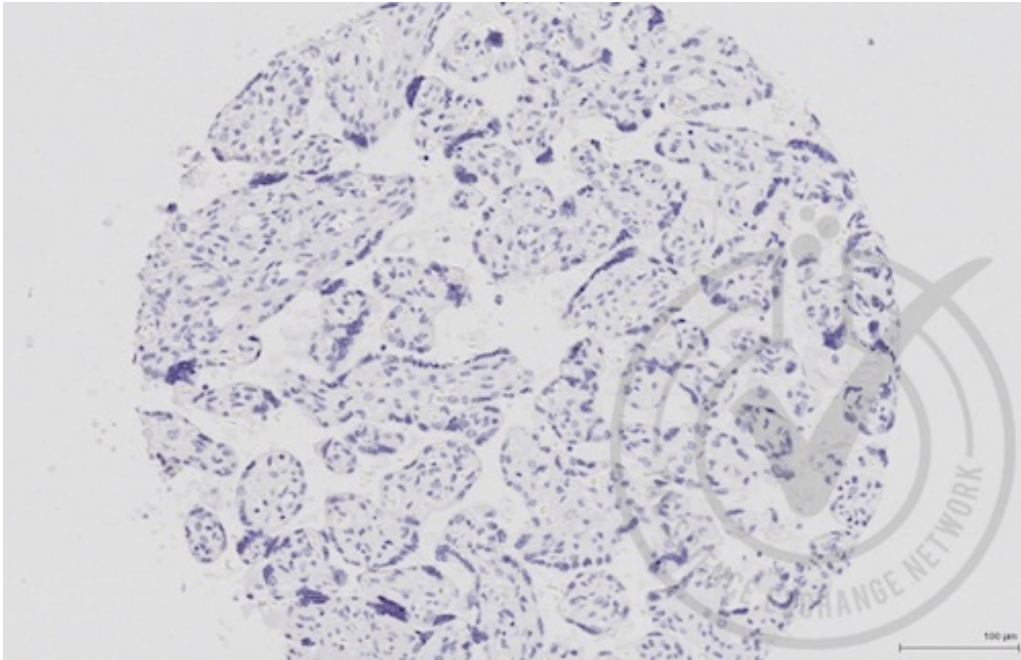


Figure 3: Human placenta tissue stained with isotype control antibody (brown) and counterstained with hematoxylin.

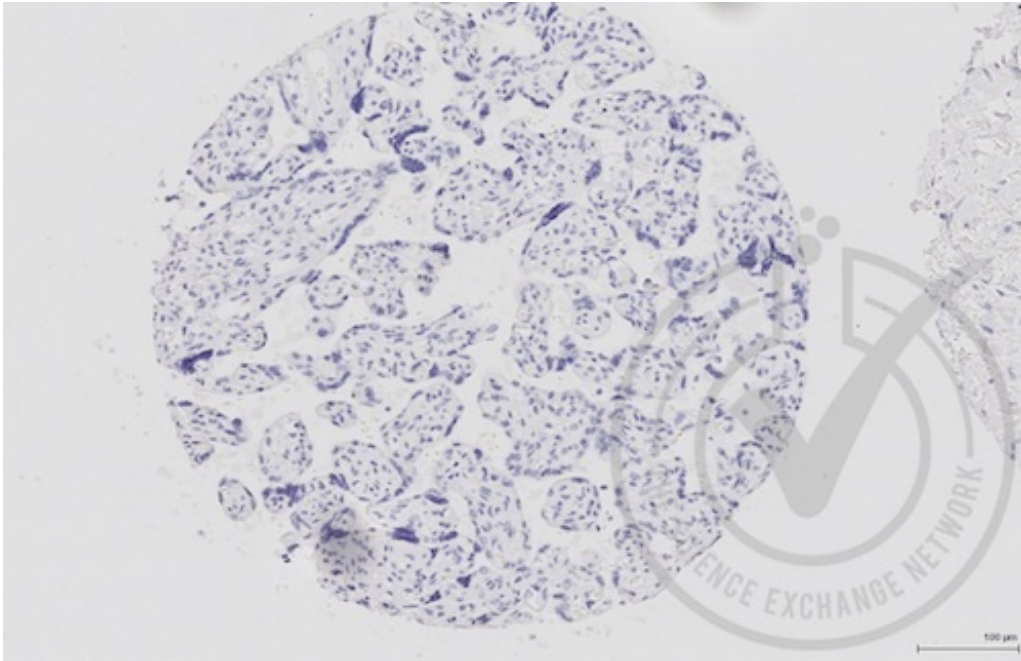


Figure 4: Human placenta tissue stained with secondary antibody only (brown) and counterstained with hematoxylin.