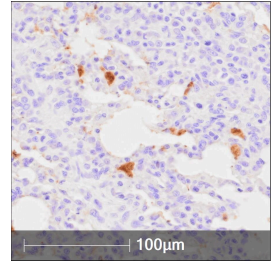




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Immunohistochemical detection of porcine reproductive and respiratory syndrome virus (PRRSV) in formalin-fixed, paraffin-embedded (FFPE) pig tissues

Forked from a private protocol



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We use this protocol and it's working

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Abstract

An immunohistochemistry (IHC) staining protocol for *in situ* identification of porcine reproductive and respiratory syndrome virus (PRRSV) in formalin-fixed, paraffin-embedded (FFPE) tissue.

Attachments



[IHC_Pig_PRRSV_Manual.](#)

⋮

414KB



Guidelines

Assay Controls

- Controls will be required to ensure there is minimal/no cross-reactivity with off-target epitopes in the tissue and to ensure stains are specific to the intended antibody targets
- IHC controls:
 - o Secondary only control
 - This slide receives only diluent in place of primary antibody. All other reagents are still applied to the slide.

Assay Variations

- Parameters may need to be further optimized for different experiments, tissues, targets, or species.



Materials

Equipment

- Pipettes/pipette tips
- Drying oven (able to reach & hold 60°C)
- Fume hood
- Slide staining tray (e.g. Simport M920-2)
- EZ-Batch Slide Holder (Advanced Cell Diagnostics 321716)
- EZ-Batch Wash Tray (Advanced Cell Diagnostics 321717)
- Tissue-Tek Vertical 24 slide rack (American Master Tech Scientific LWS2124)
- Tissue-Tek Staining Dishes (American Master Tech Scientific LWS20WH)
- Tissue-Tek Clearing Agent Dishes, xylene resistant (American Master Tech Scientific LWS20GR)
- Brightfield microscope and/or slide imaging platform

Reagents/Supplies

- Distilled water (obtained in-house)
- Phosphate-buffered saline (PBS), pH 7.2-7.4 (made in-house)
- 0.05% PBS-Tween (PBS-T), pH 7.35 (made in-house)
- Xylenes (Macron Fine Chemicals 8668-16)
- 100% ethanol (Pharmco 111000200)
 - o Dilute with distilled water to make 95%, 85%, and 70% concentrations
- Pro-Par Clearant (Anatech 510)
- Fixative
 - o 10% NBF (Cancer Diagnostics, Inc. 111) or 4% PFA (Electron Microscopy Sciences 15713)
- ImmEdge Hydrophobic Barrier Pen (Vector H-4000)
- Ready to use Proteinase-K (Dako S302080-2/S302030-2)
- BLOXALL Endogenous Blocking Solution (Vector SP-6000)
- ImmPRESS-VR Horse Anti-Mouse IgG Polymer Detection Kit, Peroxidase (Vector MP-6402-15)
 - o Normal Horse Serum, 2.5%
 - o ImmPRESS-VR Horse Anti-Mouse IgG Polymer Reagent
- Mouse anti-PRRSV monoclonal antibody clone SR30; stock concentration 0.2 g/L (RTI Lab SR30-A)
- 1% bovine serum albumin (BSA) in PBS (made in-house)
- ImmPACT DAB EqV Substrate Kit, Peroxidase (Vector SK-4103)
 - o ImmPACT DAB EqV Reagent 1 (Chromogen)
 - o ImmPACT DAB EqV Reagent 2 (Diluent)
- Gill's Hematoxylin I (American Master Tech Scientific HXGHE1LT)
- Refrax Mounting Medium (Anatech 711)
- #1 thickness cover glass (e.g. Fisherbrand 12-545-F)



Safety warnings

For all reagents, refer to MSDS to determine appropriate precautions, personal protective equipment (PPE), and disposal methods before use

Before start

Starting specimens

Starting samples = FFPE tissues cut to 4 micron thickness and adhered to positively-charged microscopy slides (e.g. SuperFrost Plus Slides; Fisher Scientific 12-550-15). It is crucial that tissues are adequately fixed to prevent tissue degradation. Tissues no thicker than 0.5 centimeters should be freshly harvested and placed into 10% neutral-buffered formalin (NBF) or 4% paraformaldehyde (PFA) at a ratio of at least 20 volumes fixative per one volume tissue. Fix tissues between 16-30 hours at room temperature (RT), followed by immediate transfer to 70% ethanol and processing into FFPE tissue blocks. Fixation times should be optimized for individual tissues and experiments.



Baking

20m

1 Before starting the assay:

- Preheat a dry oven to 60°C
- Load slides for assay into vertical slide rack

2 • **Bake slides 20 min 60°C** ⌚ 00:20:00

20m

2.1 While slides bake:

- o Prepare 0.05% PBS-T

2.2 Immediately before deparaffinizing:

- o Add ~200 mL xylenes to each of three clearing agent dishes in a fume hood
- o Add ~200 mL 100% ethanol to each of two staining dishes in a fume hood
- o Add ~200 mL 95% ethanol to a staining dish in a fume hood
- o Add ~200 mL 85% ethanol to a staining dish in a fume hood
- o Add ~200 mL 70% ethanol to a staining dish in a fume hood
- o Add ~200 mL distilled water to a staining dish in a fume hood
- o Add ~200 mL PBS-T to a staining dish

Deparaffinizing & Rehydrating

20m

3 • Submerge slide rack in fresh **xylenes 5 min RT** ⌚ 00:20:00

20m

- Submerge slide rack in fresh **xylenes 5 min RT**
- Submerge slide rack in fresh **xylenes 5 min RT**
- Submerge slides rack in fresh **100% ethanol 1 min RT**
- Submerge slides rack in fresh **100% ethanol 1 min RT**
- Submerge slides rack in fresh **95% ethanol 1 min RT**
- Submerge slides rack in fresh **85% ethanol 1 min RT**
- Submerge slides rack in fresh **70% ethanol 1 min RT**
- Submerge slides rack in fresh **distilled water 3 min RT**
- Submerge slides rack in fresh **PBS-T for transport**

3.1 While slides deparaffinize/rehydrate:

- o Turn off dry oven
- o Prepare humidified slide staining tray by adding water to bottom & placing lid on top
- o Add ~200 mL PBS-T to each of two wash trays

Hydrophobic Barrier

20m



- 4
- **Apply hydrophobic barrier** around each tissue  00:20:00 20m
 - o One by one, unload slides from vertical rack submerged in PBS-T. Dry off only the area around the tissue where a barrier will be drawn with a hydrophobic barrier pen. Keep tissue area wet the whole time. Draw barrier and place slides into the EZ-Batch slide holder placed inside the slide staining tray. Using a pipette, apply a small amount of PBS-T within the barrier (just enough to keep the tissue wet while drawing barriers on remaining slides). Slides will remain locked in the EZ-Batch slide holder throughout the protocol until being transferred back to a vertical rack for counterstaining.
 - Leave slide holder in slide staining tray

Proteinase Digestion 10m

- 5
- Decant slide holder and again place flat in slide staining tray  00:10:00 10m
 - Incubate with **Dako Ready to use Proteinase-K 3 min RT**
 - o Apply drops to completely cover tissues
 - o Let incubate in slide staining tray with lid closed
 - Decant slide holder and transfer to wash trays for PBS-T washes
 - Submerge slide holder in fresh **PBS-T 2 min RT**
 - Submerge slide holder in fresh **PBS-T 2 min RT**
- 5.1 While slides incubate with Proteinase:
- o Discard deparaffinizing & rehydrating reagents
 - o Add ~200 mL PBS-T to each of two wash trays

Tissue Quenching 15m

- 6
- Decant slide holder and again place flat in slide staining tray  00:15:00 15m
 - Incubate with **BLOXALL Endogenous Blocking Solution 10 min RT**
 - o Apply to completely cover tissues; let incubate in slide staining tray with lid closed
 - Decant slide holder and transfer to wash trays for PBS-T washes
 - Submerge slide holder in fresh **PBS-T 2 min RT**
 - Submerge slide holder in fresh **PBS-T 2 min RT**
- 6.1 While slides incubate with enzyme block:
- o Discard epitope retrieval reagents
 - o Add ~200 mL PBS-T to each of two wash trays

Protein Blocking 30m



- 7
- Decant slide holder and again place flat in slide staining tray ⌚ 00:30:00
 - Incubate with **Normal Horse Serum 2.5% 30 min RT**
 - Serum is a component of the secondary antibody reagent kit
 - Apply to completely cover tissues; let incubate in slide staining tray with lid closed
- 7.1 While slides incubate with serum:
- Discard tissue quenching reagents
 - Prepare diluted primary antibody by adding antibody stock to 1% BSA in PBS at a dilution of 1:10,000. Total volume to use is dependent on tissue sizes. Make sure to mix reagents before pipetting. Mix diluted antibody well before use.

30m

Primary Antibody

5m

- 8
- Decant slide holder and again place flat in slide staining tray ⌚ Overnight
 - Do not wash with PBS-T between decanting serum and applying secondary antibody
 - Incubate with **diluted primary antibody 4C overnight**
 - Apply to completely cover tissues; let incubate in slide staining tray with lid closed
 - • Decant slide holder and transfer to wash trays for PBS-T washes
 - Submerge slide holder in fresh **PBS-T 2 min RT**
 - Submerge slide holder in fresh **PBS-T 2 min RT**
- 8.1 While slides incubate with primary antibody:
- Discard protein blocking reagents
- The Next Day:
- Add ~200 mL PBS-T to each of two wash trays

5m

Secondary Antibody

35m

- 9
- Decant slide holder and again place flat in slide staining tray ⌚ 00:35:00
- Incubate with **Horse anti Mouse IgG (HRP-conjugated polymer) 30 min RT**
 - Secondary antibody polymer is a component of the secondary antibody reagent kit
 - Apply to completely cover tissues; let incubate in slide staining tray with lid closed
 - Decant slides and transfer to vertical slide rack for PBS-T washes
 - Submerge slide rack in fresh **PBS-T 2 min RT**
 - Submerge slide rack in fresh **PBS-T 2 min RT**

35m



- 9.1 While slides incubate with secondary antibody:
- o Discard primary antibody reagents
 - o Add ~200 mL PBS-T to each of two wash trays
- 9.2 Immediately before chromogen detection:
- o Prepare diluted DAB chromogen by adding equal volumes of DAB Reagent 1 (Chromogen) and DAB Reagent 2 (Diluent) together. Total volume to use is dependent on tissue sizes. Make sure to mix reagents thoroughly. Store in the dark due to light sensitivity.

Chromogenic Detection (DAB)

10m

- 10
- Decant slide holder and again place flat in slide staining tray 🕒 00:10:00
 - Incubate with diluted **DAB chromogen 5 min RT**
 - o Apply to completely cover tissues; let incubate in slide staining tray with lid closed
 - Decant slide holder and transfer to vertical slide rack for PBS-T washes
 - Submerge slide rack in fresh **PBS-T 2 min RT**
 - Submerge slide rack in fresh **PBS-T 2 min RT**

10m

- 10.1 While slides incubate with chromogen:
- o Discard remaining secondary antibody reagents
 - o Add ~200 mL PBS-T to each of two wash trays
 - o Add ~200 mL hematoxylin to one staining dish
 - o Add ~200 mL distilled water to each of three staining dishes

Counterstaining

2m

- 11
- Transfer slides to vertical slide rack 🕒 00:02:00
 - o Do quickly to avoid drying out slides or alternatively place vertical slide rack in a staining dish containing PBS-T and then transfer slides
 - Submerge slide rack in **hematoxylin 1 min RT**
 - Submerge slide rack in fresh **distilled water, dunking 3-5 times**
 - Submerge slide rack in fresh **distilled water, dunking 3-5 times**
 - Submerge slide rack in fresh **distilled water, dunking 3-5 times**
 - o Water should no longer appear purple in the third water dish used

2m

Mounting

45m

- 12
- Submerge slide rack in fresh **95% ethanol 1 min RT** 🕒 00:45:00
 - Submerge slide rack in fresh **100% ethanol 1 min RT**

45m

- Submerge slide rack in fresh **100% ethanol 1 min RT**
- Submerge slide rack in fresh **100% ethanol 1 min RT**
- Submerge slide rack in fresh **Pro-Par 5 min RT**
- Submerge slide rack in fresh **Pro-Par 5 min RT**
- Submerge slide rack in fresh **Pro-Par 5 min RT**
- **Mount slides** by adding 2-4 drops of mounting media to each slide, followed by application of a cover glass. Remove bubbles from tissue by applying pressure to cover glass.
- Place slides flat in a dry, dark space to air dry at RT overnight
- Assess staining with a brightfield microscope

12.1 While slides are air drying:

- o Discard chromogen detection, counterstaining, and slide mounting reagents

Protocol references

- Staining protocol was developed by Dr. Jayne Wiarda
- Staining protocol was optimized and executed by Dr. Jayne Wiarda and Colin Stoy
- We thank Adrienne Shircliff for slide sectioning and imaging