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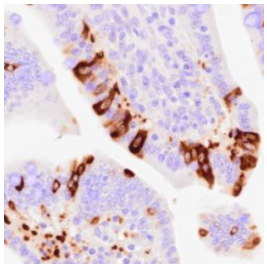
# Immunohistochemical detection of porcine epidemic diarrhea virus (PEDV) in formalin-fixed, paraffin-embedded (FFPE) pig tissues



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 epidemic diarrhea virus (PEDV) in formalin-fixed, paraffin-embedded (FFPE) tissue.

## Attachments

PDF

IHC_Pig_PEDV_ManualA...

4.3MB

## Guidelines

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| | Antibody | Undiluted | 100ug/ml | 10ug/ml | 1ug/ml | 0.1ug/ml | 0.01ug/ml | 0.001ug/ml |
| | MPEDV-5A-2F7 | 3.6 mg/ml | 1:36 | 1:360 | 1:3.600 | 1:36,000 | 1:3600,000 | 1:3,600,000 |
| | MPEDV-5A-3D6 | 5.4 mg/ml | 1:54 | 1:540 | 1:5,400 | 1:54,000 | 1:540,000 | 1:5,400,000 |
| | MPEDV-5A-5B9 | 2.62 mg/ml | 1:26.2 | 1:262 | 1:2,620 | 1:26,200 | 1:262,000 | 1:2,620,000 |
| | MPEDV-5A-6H10 | 1.95 mg/ml | 1:19.5 | 1:195 | 1:1,950 | 1:19,500 | 1:195,000 | 1:1,950,000 |
| | MPEDV-5A-7A12 | 1.75 mg/ml | 1:17.5 | 1:175 | 1:1,750 | 1:17,500 | 1:175,000 | 1:1,750,000 |

Primary antibody dilutions

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:
  - 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)
  - Dilute with distilled water to make 95%, 85%, and 70% concentrations
- Pro-Par Clearant (Anatech 510)
- Fixative
  - 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)
  - Normal Horse Serum, 2.5%
  - ImmPRESS-VR Horse Anti-Mouse IgG Polymer Reagent
- Mouse anti-PEDV antibody
  - Clone #2F7 (Immunology Consultants Laboratory MPEDV-5A-2F7, stock concentration 3.6mg/mL)
  - Clone #3D6 (Immunology Consultants Laboratory MPEDV-5A-3D6, stock concentration 5.4mg/mL)
  - Clone #5B9 (Immunology Consultants Laboratory MPEDV-5A-5B9, stock concentration 2.62mg/mL)
  - Clone #6H10 (Immunology Consultants Laboratory MPEDV-5A-6H10, stock concentration 1.95mg/mL)
  - Clone #7A12 (Immunology Consultants Laboratory MPEDV-5A-7A12, stock concentration 1.75mg/mL)
- 1% bovine serum albumin (BSA) in PBS (made in-house)
- ImmPACT DAB EqV Substrate Kit, Peroxidase (Vector SK-4103)
  - ImmPACT DAB EqV Reagent 1 (Chromogen)
  - 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
- 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**

20m

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 30m

• 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.

## Primary Antibody

5m

8 5m
- 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

## Secondary Antibody

35m

9 35m

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**

9.1 While slides incubate with secondary antibody:
- Discard primary antibody reagents
- 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) 12m

10 12m

- Decant slide holder and again place flat in slide staining tray 00:12:00
- Incubate with diluted **DAB chromogen 7 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**

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 2m

- 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

## Mounting 45m

12 45m

- Submerge slide rack in fresh **95% ethanol 1 min RT** 00:45:00
- 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 **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:
- 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, Eraldo Zanella, and Colin Stoy
· We thank Adrienne Shircliff for slide sectioning and imaging