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Manual Basescope Duplex Assay

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We use this protocol and it's working

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Abstract

This protocol details the manual basescope duplex assay.

Materials

- BaseScope™ Duplex Reagent Kit **Advanced Cell Diagnostics Catalog #**323800

Assign C1 green probe to morphology.

DAY 1

- Fresh Xylene
- 100% EtOH
- Hydrogen peroxide, Protease III
- Target Retrieval
- 25 mL 10X Target Retrieval + 225 mL Nanopure Water (TV = 250 mL)
- ImmEdge Hydrophobic Barrier Pen **Advanced Cell Diagnostics Catalog #**310018
- Nanopure water
- Probes (Green/C1 Morphology, Red/C2 Ercc1)
- 1X Wash Buffer
  - Warm 50X Buffer to 40 °C (00:10:00 - 00:20:00)
  - 40 mL 50X Buffer + 1960 mL Nanopure Water (TV=2 L)
- SSC
  - 60 mL 20X SSC + 180 mL Nanopure Water (TV = 240 mL)

DAY 2

- AMP1 – AMP12
- Wash Buffer
- Red-B
- Red-A
- Green-B
- Green-A
- Hematoxylin (MHS16)
  - 100 mL Gill's Hemotoxylin + 100 mL Nanopure water (TV= 200 mL)
- Vector Labs Vectamount (60mL) **Advanced Cell Diagnostics Catalog #**321584
- 0.02% ammonia water
  - 143 µL 28% ammonium hydroxide + 200 mL Nanopure Water (TV = 200 mL)
- Fresh Xylene

Day 1

1 Bake slides @ 60 °C for 01:00:00 . 1h

1.1 After baking, wet humidifying paper, warm oven and slide tray to 40 °C .

2 Deparaffinize

2.1 Xylene – 00:05:00 **w/ agitation**. 5m

- **Meanwhile…** turn steamer on, make 1X Target Retrieval solution, turn slide warmer to 60 °C .

2.2 Fresh Xylene – 00:05:00 **w/ agitation**. 5m

2.3 100% EtOH – 00:02:00 **w/ agitation**. 2m

2.4 Fresh 100% EtOH – 00:02:00 **w/ agitation**. 2m

2.5 Dry on slide warmer – 60 °C for 00:05:00 . 5m

3 Hydrogen Peroxide

3.1 Cover tissue with hydrogen peroxide and incubate covered for 00:10:00 . 10m

- **Meanwhile…** Boil 1X Target Retrieval solution in microwave then place in steamer.

3.2 Tap solution off on absorbent paper.

3.3 Wash in nanopure water (up and down 3-5 times then incubate for 2 min) **2X**. 4m

1. Wash in nanopure water (up and down 3-5 times then incubate for 00:02:00) (1/2).
2. Wash in nanopure water (up and down 3-5 times then incubate for 00:02:00) (2/2).

4 Target Retrieval

4.1 Transfer samples to 99 °C 1X Target Retrieval solution for 00:30:00 . 30m

4.2 Wash in nanopure water (up and down 3-5 times then incubate for ~ 00:00:15) 15s

4.3 Transfer to second container of 100% EtOH in fume hood for 00:03:00 . 3m

4.4 Dry on slide warmer – 60 °C for 00:05:00 . 5m

5 Hydrophobic Barrier

5.1 Create barrier with hydrophobic pen – let dry for 00:05:00 @ Room temperature . 5m

- **Possible stopping point: Dry O/N @** Room temperature **.**

6 Protease III

6.1 Cover tissue with Protease III for mouse or cell pellets. Protease IV for human tissues.

6.2 Incubate for 00:30:00 @ 40 °C . 40m

- Meanwhile… Warm probes to 40 °C inside oven for ~ 00:10:00 .

6.3 Wash in nanopure water w/ slight agitation **2X**.

7 Hybridize Probes

7.1 Cover tissue (~ 120 µL) with 1X probe mix (see below).

1. C1 probes are 1X; C2 probes are 50X.

- Dilute C2 probe using C1 probes to 1X.

7.2 Incubate for 02:00:00 @ 40 °C . 2h

8 Wash

8.1 Wash in 1X Wash buffer for 00:02:00 2X. 2m

- **Store O/N in 5X SSC @** Room temperature **.**

Day 2 4h 6m

9 *Turn on oven to* 40 °C *. Spray everything with RNase Away.* 4m

- Wash slides in 1X Wash buffer for **2 min 2X.**

1. Wash slides in 1X Wash buffer for 00:02:00 (1/2).
2. Wash slides in 1X Wash buffer for 00:02:00 (2/2).

10 Cover tissue with **AMP1** – incubate for 00:30:00 @ 40 °C . 34m

- Wash slides in 1X Wash buffer for **2 min 2X.**

1. Wash slides in 1X Wash buffer for 00:02:00 (1/2).
2. Wash slides in 1X Wash buffer for 00:02:00 (2/2).

11 Cover tissue with **AMP2** – incubate for 00:30:00 @ 40 °C . 34m

- Wash slides in 1X Wash buffer for **2 min 2X.**

1. Wash slides in 1X Wash buffer for 00:02:00 (1/2).
2. Wash slides in 1X Wash buffer for 00:02:00 (2/2).

12 Cover tissue with **AMP3** – incubate for 00:15:00 @ 40 °C . 19m

- Wash slides in 1X Wash buffer for **2 min 2X.**

1. Wash slides in 1X Wash buffer for 00:02:00 (1/2).
2. Wash slides in 1X Wash buffer for 00:02:00 (2/2).

13 Cover tissue with **AMP4** – incubate for 00:30:00 @ 40 °C . 34m

- Wash slides in 1X Wash buffer for **2 min 2X.**

1. Wash slides in 1X Wash buffer for 00:02:00 (1/2).
2. Wash slides in 1X Wash buffer for 00:02:00 (2/2).

14 Cover tissue with **AMP5** – incubate for 00:30:00 @ 40 °C . 34m

- Wash slides in 1X Wash buffer for **2 min 2X.**

1. Wash slides in 1X Wash buffer for 00:02:00 (1/2).
2. Wash slides in 1X Wash buffer for 00:02:00 (2/2).

15 Cover tissue with **AMP6** – incubate for 00:15:00 @ 40 °C . 19m

- Wash slides in 1X Wash buffer for **2 min 2X.**

1. Wash slides in 1X Wash buffer for 00:02:00 (1/2).
2. Wash slides in 1X Wash buffer for 00:02:00 (2/2).

16 **AMP 7 & 8 are @** Room temperature

34m

Cover tissue with **AMP7** – incubate for 00:30:00 @ Room temperature .

- Wash slides in 1X Wash buffer for **2 min 2X.**

1. Wash slides in 1X Wash buffer for 00:02:00 (1/2).
2. Wash slides in 1X Wash buffer for 00:02:00 (2/2).

17 Cover tissue with **AMP8** – incubate for 00:15:00 @ Room temperature .

19m

- Wash slides in 1X Wash buffer for **2 min 2X.**

1. Wash slides in 1X Wash buffer for 00:02:00 (1/2).
2. Wash slides in 1X Wash buffer for 00:02:00 (2/2).

18 Detect Red signal

18.1 Make enough solution for each sample.

2 µL Red-B + 120 µL Red-A = 1 sample (**Light sensitive**).

18.2 Cover tissue with solution – incubate for 00:10:00 @ Room temperature .

10m

18.3

4m

- Wash slides in 1X Wash buffer for **2 min 2X.**

1. Wash slides in 1X Wash buffer for 00:02:00 (1/2).
2. Wash slides in 1X Wash buffer for 00:02:00 (2/2).

19 **AMP 9 & 10 @** 40 °C .

19m

Cover tissue with **AMP9** – incubate for 00:15:00 @ 40 °C .

- Wash slides in 1X Wash buffer for **2 min 2X.**

1. Wash slides in 1X Wash buffer for 00:02:00 (1/2).

2. Wash slides in 1X Wash buffer for 00:02:00 (2/2).

20 Cover tissue with **AMP10** – incubate for 00:15:00 @ 40 °C . 19m

- Wash slides in 1X Wash buffer for **2 min 2X.**

1. Wash slides in 1X Wash buffer for 00:02:00 (1/2).
2. Wash slides in 1X Wash buffer for 00:02:00 (2/2).

Turn off Oven!!!! Rest of protocol is at Room temperature **.**

21 Cover tissue with **AMP11** – incubate for 00:30:00 @ Room temperature . 34m

- Wash slides in 1X Wash buffer for **2 min 2X.**

1. Wash slides in 1X Wash buffer for 00:02:00 (1/2).
2. Wash slides in 1X Wash buffer for 00:02:00 (2/2).

22 Cover tissue with **AMP12** – incubate for 00:15:00 @ Room temperature . 19m

- Wash slides in 1X Wash buffer for **2 min 2X.**

1. Wash slides in 1X Wash buffer for 00:02:00 (1/2).
2. Wash slides in 1X Wash buffer for 00:02:00 (2/2).

23 Detect Green Signal

23.1 Make enough solution for each sample.

- 2.4 µL Green-B + 120 µL Green-A = 1 sample **(Light sensitive)**.

23.2 Cover tissue with solution – incubate for 00:10:00 @ Room temperature . 10m

23.3 Wash slides in 1X Wash buffer for 00:05:00 . 5m

23.4 Rinse slides in nanopure water.

24 Counterstain: if hematoxylin is too dark, it will mask signal; especially green).

24.1 Incubate slides in 50% hematoxylin staining solution for 00:00:30 – 00:01:00 . 1m 30s

- Slides should turn purple.

24.2 Take out immediately and wash in nanopure water **3-5X**.

- Until slides are clear.

24.3 Dip slides up and down 2-3X in 0.02% ammonia water (should turn blue).

24.4 Wash in nanopure water **3-5X**.

25 Dry Slides. 15m

Dry for 00:15:00 @ 60 °C .

26 Mounting.

26.1 Dip quickly in FRESH xylene

26.2 Place 1-2 drops of VectaMount on the slide.

26.3 Coverslip.

26.4 Air-dry.

Protocol references

Refer to ACD manual Basescope duplex assay for reference.