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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 Hematoxylin +  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 @

5m

 Room temperature .



▪ **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 μ L Red-B +  120 μ L Red-A = 1 sample (**Light sensitive**).

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

10m



18.3 ▪ Wash slides in 1X Wash buffer for **2 min 2X**.

4m

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.