STORE

SOP Title:

HER2:HER proximity ligation assay on formalin fixed paraffin embedded tissue using the DUOLINK system

The STORE processing methods were shown to be fit-for purpose for DNA, RNA and protein extraction from FFPE material. The STORE characterisation methods were shown to be fit for-purpose for Quality Control of the extracted DNA, RNA and proteins.

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Title: HER2:HER proximity ligation assay on formalin fixed paraffin embedded tissue using the DUOLINK system

Version 1.0

Author: Melanie Spears **Issue Date: September 2010**

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Revision History

Date of Review:

Comments:

Purpose & Scope:

- This Standard Operating Procedure (SOP) describes the procedure used for carrying out the HER2:HER3 proximity ligation assay (PLA) from formalin-fixed paraffin embedded (FFPE) tumour samples using the Cambridge Bioscience PLA kits .
- The purpose of the document is to ensure that the HER2:HER3 PLA from FFPE tissue is performed to a consistently high standard.
- This SOP applies to all staff who carrying out HER2:HER3 PLAs from FFPE tissue within the Edinburgh Cancer Research Centre.

Responsibilities:

- All staff should read and sign off any related risk assessment and COSHH documents before proceeding with the method outlined in this SOP.

- All staff who carries out HER2:HER3 PLA in FFPE tissue should follow the procedures outlined in this document

- The Research Manager and/or Senior staff are responsible for ensuring this document is correct and for ensuring that any amendments are written up.

Documents:

- ECRC/ECG/Lab/Gen/024 Waste disposal procedures
- ECRC/ECG/Lab/Gen/026 General Laboratory Procedures

Document Amendment Form:

Number	Date	Page No.	Amendment	Authorised by
1				
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N.B.

1. The amendment must be authorised by the relevant senior staff.
2. The amendment must be underlined and an asterisk written in the margin alongside the change. Liquid paper **must not** be used.
3. Five or less minor amendments can be made before the procedure is revised.
4. Major changes must result in the immediate review of the procedure.

Materials

- HER2 Antibody DAKO (A0485)
- HER3 Antibody Neomarkers (MS-201-P1)
- PLA Probe 100 Mouse minus OLINK (90201)
- PLA Probe 100 Rabbit plus OLINK (90302)
- PLA Duolink Detection Kit 613 (90103)

1.0 Dewax and re-hydrate

1. Xylene 10 mins
2. Xylene 10mins
3. 99% ethanol 2 mins
4. 99% ethanol 2 mins
5. 95% ethanol 2 mins
6. 70% ethanol 2mins
7. H_2O prior to antigen retrieval

2.0 Antigen retrieval

8. Preheat Citrate buffer pH6.0 to 95-99 ºC in coplin jar in water bath
9. After the slides are dewaxed and rehydrated, immerse slides in preheated citrate buffer and incubate for 40 mins
10. Remove coplin jar from water bath and allow to cool for 20 mins at room temperature
11. Remove slides to coplin jar with TBS, and add 3-4 drops 1M Glycine pH8.5 with Pasteur pipette
12. Incubate 5 mins room temp
13. Replace TBS/Glycine solution
14. Incubate 5 mins room temp
15. Replace with TBS solution

***Note*:** All wash steps should be preformed in a coplin jar on a shaker with gentle agitation

3.0 Blocking

16. Delimit samples with hydrophobic pen. Use the reaction volume guide (Art. No. 80520) provided in the DUOLINK user manual to decide a volume suitable for the reaction.

17. Prepare 1 x Blocking solution. Dilute the Duolink Blocking stock 1:50 in high purity water.
18. Add Blocking Solution to each sample
19. Incubate 37 ºC 30 mins in a humidity chamber

4.0 Primary Antibody Incubation

20. Mix and dilute primary antibodies at suitable concentration in 1 x Antibody Diluent. Use the HER2 antibody at 1:500, HER3 at 1:200 and the antibody diluent at 1:5.
21. Tap off Blocking solution
22. Immediately add primary antibody solution to each sample.
23. Incubate 4 ºC overnight in a humidity chamber

5.0 PLA probes

24. Mix and dilute the two PLA probes (mouse minus and rabbit plus) and and antibody diluent 1:5.
25. Tap off primary antibody solution from slides
26. Wash slides in TBS-T for 5 mins room temp
27. Repeat wash step
28. Add PLA probe solution to each sample
29. Incubate 37 ºC 2 hours in a humidity chamber

6.0 Hybridisation

30. Prepare 1 x Duolink Hybridisation solution by diluting 1:5 with high purity water
31. Tap off PLA probe solution
32. Wash slides in TBS-T for 5 mins room temp
33. Repeat wash step
34. Add Hybridisation solution to each sample
35. Incubate 37 ºC 15 mins in a humidity chamber

7.0 Ligation

36. Prepare 1 x Duolink Ligation solution by diluting 1:5 with high purity water
37. Tap off Hybridisation solution
38. Wash slides in TBS-T for 1 min room temp
39. Add Duolink ligase to ligation solution at 1:40 dilution, vortex to mix
40. Add Ligation solution to each sample
41. Incubate 37 ºC for 15 mins in a humidity chamber

8.0 Amplification

42. Prepare 1 x Duolink Amplification solution by diluting 1:5 with high purity water
43. Tap off Ligation solution
44. Wash slides in TBS-T for 2 mins room temp
45. Repeat wash step
46. Add Duolink Polymerase to amplification solution at 1:80 dilution, vortex to mix
47. Add Amplification solution to each sample
48. Incubate 37 ºC for 90 mins in a humidity chamber

Note: light sensitive reagents – protect from light

9.0 Detection

49. Prepare 1 x Duolink Detection solution by diluting 1:5 in high purity water
50. Tap off Amplification solution
51. Wash slides in TBS-T for 2 mins room temp
52. Repeat wash step
53. Add Detection solution to each sample
54. Incubate 37 ºC 60 mins in a humidity chamber

Note: light sensitive reagents – protect from light

10.0 Wash and mount

55. 2 x SSC 2 mins
56. 1 x SSC 2 mins
57. 0.2 x SSC 2 mins
58. 0.1 x SSC 2 mins
59. 70% Ethanol 20 secs
60. Allow slides to dry
61. Mount slides in Vectashield
62. Analyse in afluorescnce microscope as soon as possible or store at -20^{o}C.

APPROVAL AND SIGN OFF:

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