Detection of seeded pathology using tyramide amplification

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

This protocol details the detection of seed pathology using tyramide amplification.

ATTACHMENTS

956-2484.docx

MATERIALS

TBST:

	A	B
	Tris-HCl pH 7.4	20 mM
	NaCl	150 mM
	Triton X 100	0.05%

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40m

1 Mount the fixed floating 40-micron sections onto gelatin-coated slides and dry at Room temperature 20m Overnight .

2 Rehydrate the slides in TBST (refer materials section) and digest with proteinase K (PK, 20 undetermined) diluted in TBST for 00:20:00 at 37 °C . 20m

3 Fix the slides in 4% paraformaldehyde for 00:20:00 , rinse 3 times in TBST, and incubate with 3% hydrogen peroxide for 00:30:00 to quench endogenous peroxidases. 50m

4 Place the slides in blocking buffer (TBST, 3% bovine serum albumin, 2% goat serum) for 01:00:00 and 2h then incubate it Overnight at 4 °C in blocking buffer containing anti-PSER129 antibody EP1536Y (Abcam) diluted 1:50,000.

5 Next day, wash the slides 3 times in TBST and incubate with biotinylated anti-rabbit antibody (Vector Labs) 1h diluted 1:400 in blocking buffer for 01:00:00.

6 Wash the slides 3 times in TBST and incubate with avidin-biotin complex (ABC) reagent (Vector labs) dilute 1h in blocking buffer for 01:00:00.

7 Wash the slides twice with borate buffer [M] 0.1 Mass Percent Sodium tetraborate pH 8.5) and incuba 30m in borate buffer containing 0.003% hydrogen peroxide and [M] 5 micromolar (µM) biotinyl tyramide (Sigma-Aldrich) for 00:30:00.

8 Wash the slides 3 times in TBST and incubate with ABC reagent for 01:00:00. 1h

9 Heating the slides for 00:30:00 at 80 °C in [M] 20 millimolar (mM) citrate buffer pH 6.0 bef 30m ABC reagent can increase detection sensitivity.

10 Wash the slides in TBST and develop using nickel-enhanced 3,3'-Diaminobenzidine DAB as previously described (refer references section).

11 Counterstain the slides with methylgreen (Sigma), dehydrate with graded alcohols, clear with xylenes, and cover the slides using cover slipps with cytoseal 60 (Fisher Scientific).

12 Perform the Brightfield microscopy using Nikon A1 laser scanning microscope.

13 Perform the density analysis including binary masks and region of interest (ROI) analyses using Elements software (Nikon).