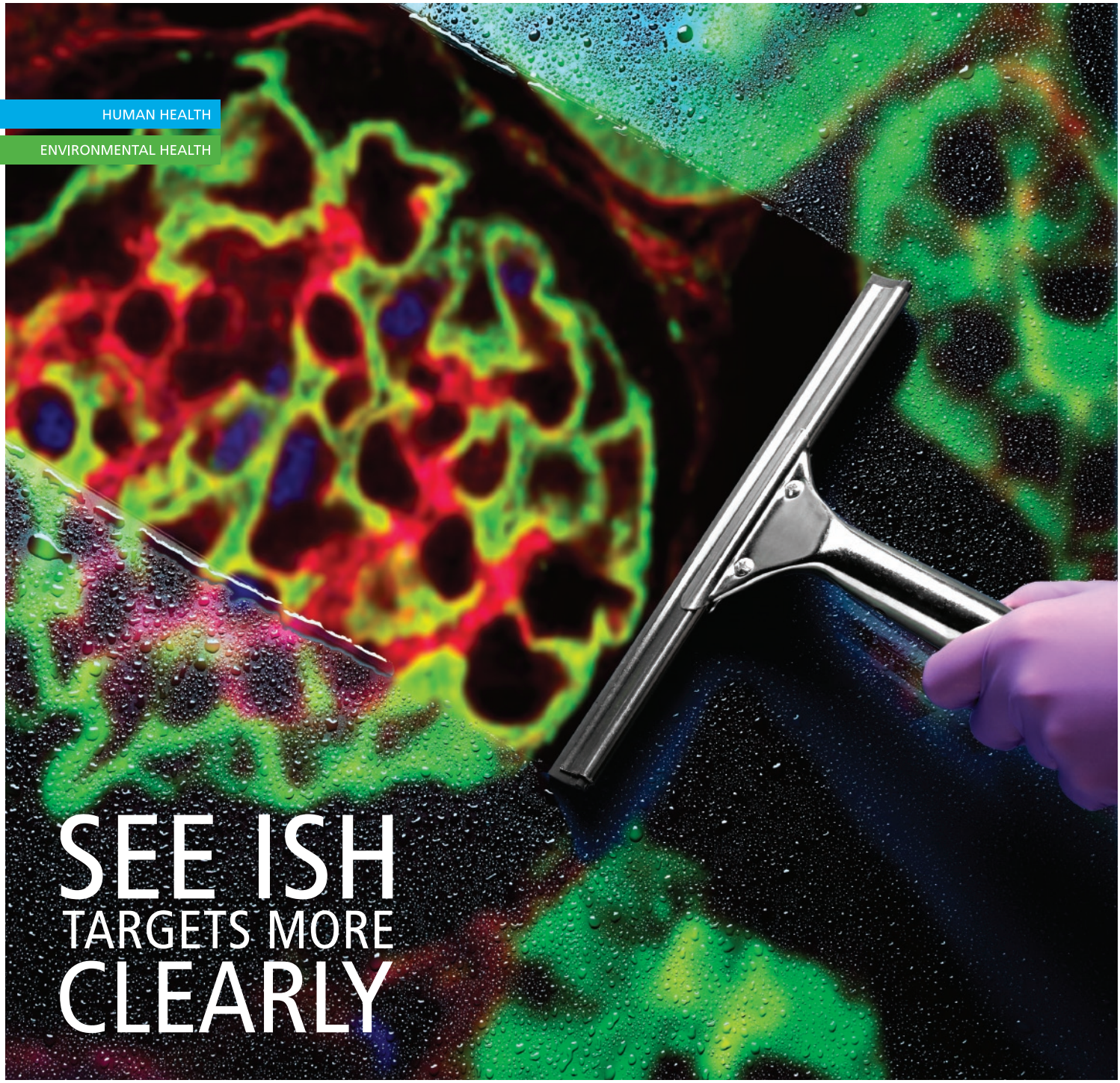
HUMAN HEALTH

ENVIRONMENTAL HEALTH



SEE ISH TARGETS MORE CLEARLY

TSA Signal Amplification for *in situ* Hybridization (ISH)

PerkinElmer®
For the Better

SEE WHAT YOU'VE BEEN MISSING

Tyramide Signal Amplification (TSA™) provides remarkable sensitivity enhancement for your ISH experiments without the drawbacks associated with other methods.

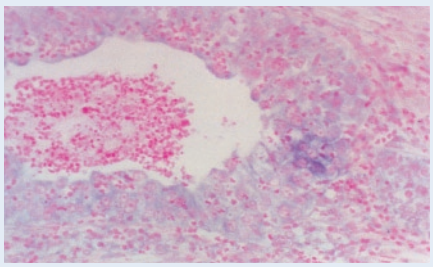
Signal amplification with TSA allows detection at levels as low as a single copy and enables the use of shorter probes for more precise localization of targets. The TSA reaction occurs within 10 minutes, and labels are bonded covalently, ensuring outstanding resolution.

- See previously undetectable weakly expressed targets
- Achieve outstanding resolution and clarity
- Reduce probe consumption while improving specificity
- Add to current protocol with minimal disruption
- Eliminate background problems with TSA's biotin-free formats
- Investigate co-localization with multi-target detection kits

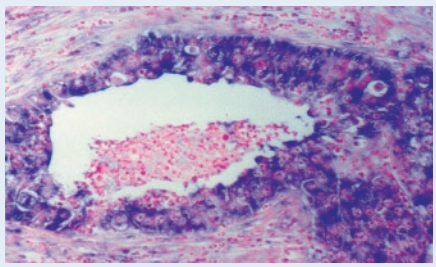
TSA is ideal for use with PerkinElmer's Cellular Imaging and Analysis solutions.

OUTSTANDING SENSITIVITY WITH HIGH RESOLUTION

TSA can increase sensitivity up to 1000-fold.



(A) Standard ISH



(B) TSA Plus ISH

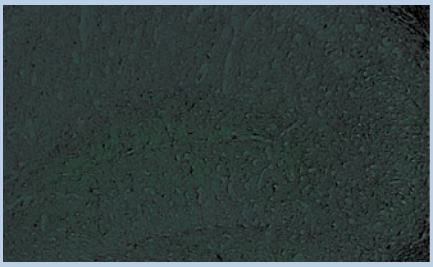
Detection of p53 mRNA in lung tissue. Digoxigenin-labeled p53 RNA probes were hybridized to paraffin-embedded lung tissue. Comparison shows (A) standard digoxigenin detection with alkaline phosphatase/BCIP-NBT (60-minute substrate incubation) and (B) TSA Plus DNP with alkaline phosphatase/BCIP-NBT (15-minute substrate incubation).

PerkinElmer R&D

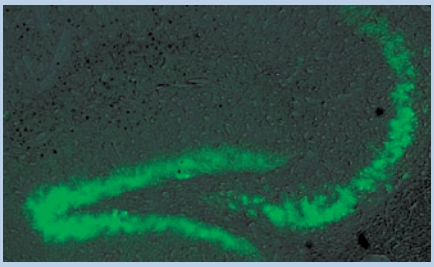
Use **TSA Plus** to enhance sensitivity 10-20-fold over standard TSA.

SHARPER IMAGES WITH CLEARER RESULTS

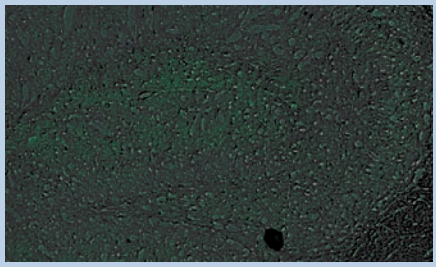
TSA delivers outstanding sensitivity without adding background



(A) Standard ISH



(B) TSA ISH



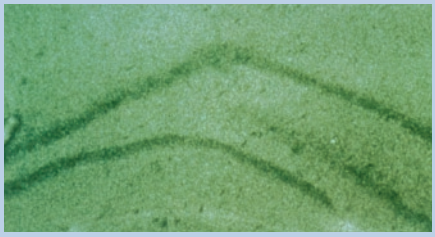
(C) TSA ISH, negative control

ISH detection of p18 gene expression in mouse brain section with a DIG-labeled probe. (A) Detection with fluorophore-labeled anti-DIG. (B) Detection with anti-DIG HRP followed by TSA Plus Fluorescein. (C) Probe omitted; same detection as (B).

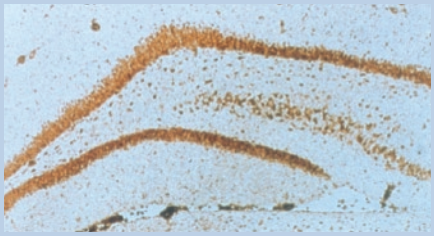
Sandrine Bichet and Brigitte Gross-Scherf, Friedrich Miescher Institute, Novartis Research Foundation

SHORTER TIME TO RESULTS

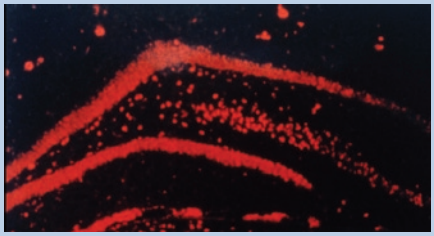
TSA delivers results in one day instead of weeks for radiometric detection



Radiometric detection, 1-month exposure.



TSA with chromogenic detection (DAB).

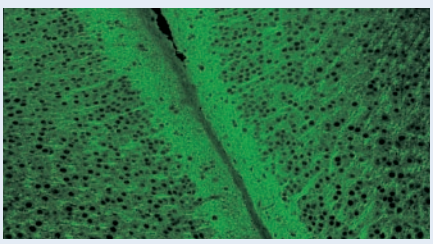


TSA fluorescent detection (TMR).

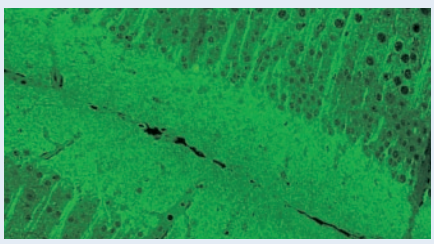
Comparison of radiometric and TSA-amplified ISH detection of muscarinic receptor mRNA in rat brain hippocampus.

PerkinElmer R&D

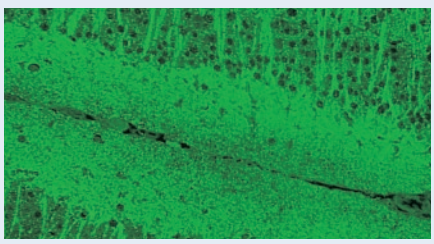
Mouse Brain, 20x magnification, 2-second exposure



Conventional detection with fluorescein conjugated 2° antibody. Dilution 1:100.



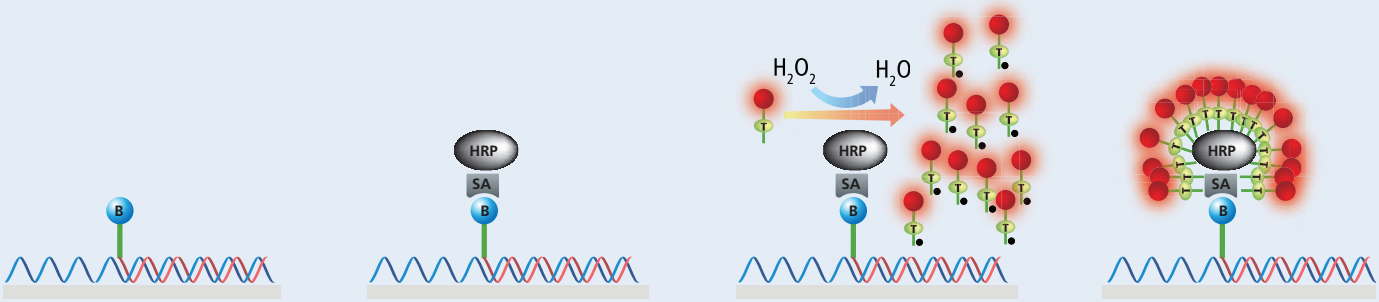
Standard TSA fluorescein. Dilution 1:10,000.



TSA Plus fluorescein. Dilution 1:1,000,000.

HOW DOES TSA WORK FOR ISH?

1. Hybridize sample with hapten labeled probe. Biotin, DIG, DNP and fluorescein have been used as labels.
2. Introduce HRP as streptavidin or antibody conjugate.
3. Incubate with TSA reagent (3-10 min). HRP catalyzes formation of TSA free radicals.
4. TSA free radicals form covalent bonds with tyrosine residues proximal to HRP. Unbound TSA radicals form dimers that are washed away.



ADDING TO *IN SITU* HYBRIDIZATION (ISH/FISH/CISH) EXPERIMENTS

Nucleic acid to detect	Labeled probe	Introduction of HRP	TSA possibilities	Detection options
	B	SA HRP	F	**Direct Fluorescence** • Coumarin (ex. 402 nm, em. 443 nm) • Fluorescein (ex. 494 nm, em. 517 nm) • TMR (ex. 550 nm, em. 570 nm) • Cyanine 3 (ex. 550 nm, em. 570 nm) • Cyanine 5 (ex. 648 nm, em. 667 nm)
	DIG DIG, FITC…	HRP HRP-conjugated secondary antibody (other options include HRP polymer conjugates, ABC)	DNP B FITC	**Chromogenic** • Streptavidin-HRP or AP plus chromogen of choice • Anti-DNP-HRP or AP with chromogen of choice • Anti-fluorescein-HRP or AP with chromogen of choice **Indirect Fluorescence** • Streptavidin-fluorophore conjugate • Anti-DNP-fluorophore conjugate • Anti-fluorescein-fluorophore conjugate

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

008636A_01 Printed in USA Sep. 2010