

Materials List for:

Detection of the Genome and Transcripts of a Persistent DNA Virus in Neuronal Tissues by Fluorescent *In situ* Hybridization Combined with Immunostaining

Frédéric Catez^{1,2,5}, Antoine Rousseau⁴, Marc Labetoulle⁴, Patrick Lomonte^{1,2,3}

¹Virus and Centromere Team, Centre de Génétique et Physiologie Moléculaire et Cellulaire, CNRS UMR 5534

²Université de Lyon 1

³Laboratoire d'excellence, LabEX DEVweCAN

⁴Institut de Virologie Moléculaire et Structurale, CNRS UPR 3296

⁵Centre de Recherche en Cancérologie de Lyon, INSERM U1052, CNRS UMR 5286

Correspondence to: Patrick Lomonte at patrick.lomonte@univ-lyon1.fr

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Materials

Name	Company	Catalog Number	Comments
Balb/c mice	Janvier, France		6 week-old females
HSV-1 strains	SC16 strain (wild type)		See Labetoulle, M. et al. <i>Invest. Ophthalmol. Vis. Sci</i> 44 , 217–225 (2003) for details on HSV-1 strain and virus stock preparation.
Ketamine hydrochloride	Sigma	K2753	Intraperitoneal injection of a solution containing Ketamine (100mg/kg) and Xylazine (10mg/kg)
Xylazine hydrochloride	Sigma	X1251	
Paraformaldehyde (PFA)	Sigma	158127	Suspend 4 g of PFA in 90 ml of water. Add 50 µl of 1N NaOH, and heat at 60 °C in a water bath with agitation. PFA dissolves in about 30 min. Add 10 ml of 10x PBS. This solution can be prepared in advance and stored at -20 °C in 5 ml tubes. Caution. Manipulate under a fume hood.
Physiological Saline	Sigma	07982-100TAB-F	
1x PBS, pH 7.4 (sterile)	Life Technologies	10010-015	
Sucrose	Sigma	84100	Prepare a 20% sucrose solution in 1x PBS.
Cryosectioning embedding medium - Tissue-Tek OCT Compound	SAKURA	4583	
Large vector DNA purification kit	Qiagen	12462	To purify Cosmid or BAC vector containing HSV-1 genome and store at -20 °C
Nick translation kit	Roche Applied Sciences	10 976 776 001	
Cy3-dCTP	GE Healthcare	PA53021	Protect from light
0.5 M EDTA	Sigma	E6758	
G50 Mini spin column	GE Healthcare	27-5330-01	
Salmon sperm DNA 10 mg/ml	Invitrogen Life Technologies	15632-011	
Ethanol molecular biology grade	Sigma	87047	Prepare a 70% solution
Salmon sperm DNA	Invitrogen Life Technologies	15632-011	

Formamid Molecular biology grade	Sigma	F9037	Caution. Manipulate under fume hood.
HSV-1 biotinylated commercial probe	Enzo Life Sciences	ENZ-40838	
ImmEdge hydrophobic pen	Vector Laboratories	H-4000	
20x Saline Sodium Citrate (SSC)	Sigma	S6639	Prepare a 2x SSC solution in ddH ₂ O.
Triton X-100	Sigma	T8787	Prepare a 10% stock solution in water and store at +4 °C. Prepare the 0.5% solution in 1x PBS right before use.
10 mM Sodium citrate pH 6.0	Sigma	S1804	Prepare a 100 mM stock solution (10x). Weigh 10.5 g of citric acid (MW 210.14). Caution, irritant and toxic, wear appropriate mask and gloves), and dissolve in 400 ml water. Adjust pH at 6.0 with 1 N NaOH (caution, irritant, wear gloves). Adjust to 500 ml with distilled water. Dilute 10x in distilled water before use.
Acetic Acid	Sigma	320099	
Methanol, molecular biology grade	Sigma	322415	
Dextran sulfate - MW 500,000	Euromedex	EU0606-A	
Denhardt's solution (100x)	Euromedex	1020-A	
Rubber Cement "FixoGum"	Marabut	290110000	
DNA purification kit - Qiaquick PCR purification kit	Qiagen	28104	
T7 <i>in vitro</i> transcription kit	Ambion Life Technologies	AM1314	
Biotin-16-UTP	Roche Applied Sciences	11388908910	
RNA purification minicolumn	Qiagen	73404	
Ribonucleoside VanadyI Complex	New England Biolabs	S1402S	
H ₂ O ₂	Sigma	H3410	Prepare a 3% solution in distilled water. Store at +4 °C and protect from light.
Yeast tRNA	Invitrogen	15401011	prepare a 10 mg/ml solution in RNase free water
Normal Goat Serum	Invitrogen	PCN5000	
Primary antibodies	any supplier		The following primary antibodies were used in the result section: anti-mouse CENP-A (rabbit mAb C51A7, Cell Signaling Technologies), and anti-ATRX H-300 (Santa Cruz Biotechnology)
Secondary fluorescent antibodies	Invitrogen Life Technologies		The fluorescent secondary antibodies routinely used in our protocol are Alexa Fluor labeled goat antibodies (IgG H+L). The antibody used in the result section is an anti-rabbit goat antibody labeled with Alexa Fluor 488 (reference A11001)
Tyramide Signal Amplification (TSA) kit - Streptavidin + Alexa Fluor 350 (blue fluorescence)	Invitrogen Life Technologies	#T20937	TSA kits are also available from Perkin Elmer

Tyramide Signal Amplification (TSA) kit - Streptavidin + Alexa Fluor 488 (green fluorescence)	Invitrogen Life Technologies	#T20932	
Hoechst 33342	Invitrogen Life Technologies	H3570	Prepare a 0.5 µg/ml solution in 1x PBS immediately before use. Discard the remaining solution.
22 mm x 50 mm coverslip. n°1.5 glass	Electron Microscopy Sciences	72204-04	
Mounting medium with anti-fading agent - VECASHIELD	Vector Laboratories	H-1000	Another conventional product is Fluoromount G from Electron Microscopy Science
Superfrost glass slides	FisherScientific	12-550-15	
Equipment/Material	Company	Reference	Note
Needle for infection			Glass micropipette hot drawn. Custom made.
Dissection equipment	Moria, France		Microsurgical scissors and forceps
Peristaltic pump	Cole Palmer Instruments		Easyload Masterflex
Microsyringe pump device (Nano Pump)	kdScientific	KDS310	
Cryostat	Leica France	CM 1510-1	
-80 °C freezer	Sanyo		Ultra Low -80 °C
Domestic microwave oven			
Dry block heater	Eppendorf	022670204	
Incubator Slide moat	Boekel Scientific	240000	
Coplin Jar	Dominique Dutscher	68512	
Staining glass container	Dominique Dutscher	68506	
Fluorescent microscope	Zeiss		The images presented in the result section were collected with a Zeiss AxioObserver with objective 40X LD NeoFluor N.A 0.6, and 100X PlanApochromat N.A 1.3. Filter set #38, #43, and #43. HXP 120 fluorescence light source. Photometrics CoolSNAP HQ2 CCD camera. Signal will be more easily observed on a recent high efficiency microscope such as Zeiss AxioImager/AxioObserver series, Nikon Ti-E/Ni-E series or Leica DM/DMI6000 series