

Materials List for:

Analysis of Single-cell Gene Transcription by RNA Fluorescent *In Situ* Hybridization (FISH)

Elena Ronander^{1,2}, Dominique C. Bengtsson^{1,2}, Louise Joergensen^{1,2}, Anja T. R. Jensen^{1,2}, David E. Arnot^{1,2,3}

¹Centre for Medical Parasitology, Department of International Health, Immunology & Microbiology, Faculty of Health Sciences, University of Copenhagen

²Department of Infectious Diseases, Copenhagen University Hospital (Rigshospitalet)

³Institute of Infection and Immunology Research, School of Biology, University of Edinburgh

Correspondence to: Elena Ronander at elenar@sund.ku.dk

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Materials

Name	Company	Catalog Number	Comments
Acetic Acid	Sigma/Aldrich	338826-100ml	
Albumax media: RPMI 1640 Glutamine solution	Lonza	BE12-115F	500 ml RPMI 1640 5 ml glutamine solution
Albumax media: Gentamycin sulphate Albumax-solution	Lonza	BE02-012E	2.5 ml gentamycin sulphate 50 ml Albumax-solution
Albumax-solution: Hypoxanthine	Sigma-Aldrich	H9377	0.8 g Hypoxanthine
Albumax-solution: AlbuMAX II	Invitrogen	11021-037	200 g Albumax
Albumax-solution: RPMI 1640	Lonza	BE12-115F	4 liter RPMI 1640 Dissolve with magnet at max. 50 °C. Filter sterilize and store at -20 °C in aliquots.
Amberlite	Sigma	A5710-110G	Resin to deionize formamide.
Anti-biotin goat pAb Peroxidase Conjugate	Calbiochem	203206	
Anti-Dig antibody	Novus biologicals	NB100-41330	
Anti-fade reagent with DAPI	Invitrogen	P36931	The mounting media has to cure for 24 hr before sealing the slide completely.
Biotin RNA Labeling Mix	Roche	11685597910	
Camera digital	Nikon digital sight DC-F11		
Coverslips	Menzel-glaser	631-1570	
culture flask 25 cm ² Nunclon surface	Nunc	156340	
DEPC	Fluka/Sigma	32490-100ml	1 ml DEPC in 1000 ml deionized water. Add a stir bar and stir for 12 hr. Autoclave for 30 min.
Digital camera	Nikon digital sight DC-F11		
Dynabeads Protein A	Invitrogen	10002D	
DynaMiq-15 Magnet	Invitrogen	123-01D	
Formamide bioultra 99 %	Fluka/Sigma	47671-1l-F	Deionized formamide: 5 g of ion exchange resin per 100 ml of formamide. Stir 30 min. Filter through Whatman paper.
Gelatine 0.75% solution: Gelatine	Sigma-Aldrich	G2500	3.75 g gelatine in 500 ml RPMI 1640. Heat to 56 °C to dissolve. Filter sterilize when 56 °C. Store at -20 °C in aliquots.

Gelatine 0.75% solution: RPMI 1640	Lonza	BE12-115F	3.75 g gelatine in 500 ml RPMI 1640. Heat to 56 °C to dissolve. Filter sterilize when 56 °C. Store at -20 °C in aliquots.
Glutamine solution: L-glutamin	Sigma-Aldrich	G3126	14.6 g L glutamine in 500 ml 0.9 % NaCl. Dissolve, filter sterilize and store at -20 °C in aliquots.
Glutamine solution: HCl	Sigma	H1758	14.6 g L glutamine in 500 ml 0.9 % NaCl. Dissolve, filter sterilize and store at -20 °C in aliquots.
Hybridization solution: Formamide Bio ultra 99% SSC 20x	Fluka/Sigma	47671-1I-F	Total 20 ml, keep frozen at -20 °C in aliquots 10 ml deionized formamide
Hybridization solution: Denhardt's 50x concentrate	Sigma	D2532	5 ml 20xSSC
Hybridization solution: Yeast tRNARoche blocking reagent	Sigma	R-6750	2 ml 50x Denhardt's 250 µl 20 mg per ml yeast tRNA
Hybridization solution: Salmon sperm DNA	Fluka/Sigma	31149-106GF	0.4 g Roche blocking reagent 1 ml of 10 mg per ml salmon sperm DNA (Critical: denature salmon spermDNA at 96 °C for 5 min before adding to the hybridization solution)
Hybridizer ThermoStar 100 HC4	Quantifoil Instruments GmbH	1004-0011	Can be replaced by hybridization oven and RNase free hybridization chambers padded with DEPC water.
Immersion oil UV transparent fluorescence free	Sigma	10976-1EA	
Immunofluorescence or confocal microscope	Nikon D-Eclipse TE2000C		
Nailpolish	Available in any drugstore		
PBS 20x:NaCl KCl Na ₂ HPO ₄ x 2H ₂ O KH ₂ PO ₄ DEPC deionized H ₂ O			Ajust pH to 7.4 160 g 4 g 23 g 4 g 1 l
Paraformaldehyde 4 %	Fluka/chemika	76240	4 g PFA in 80 ml PBS/DEPC. Heat to 65 °C until the PFA dissolves. Add 20 ml PBS, allow the solution to cool. Adjust the pH to 7.4. Filter. Store in aliquots at -20 °C.
Paraformaldehyde 4 %/ Acid acetic 5 %			950 µl paraformaldehyde + 50 µl Acetic acid
Pepsin	Sigma/Aldrich	P7000	
Peroxidase-conjugated anti-biotin	Calbiochem	203206	
RBC-wash media: RPMI 1640 Glutamine solution	Lonza	BE12-115F	500 ml RPMI 1640 5 ml glutamine solution
RBC-wash media: Gentamycin sulfate	Lonza	BE02-012E	2.5 ml gentamycin sulphate
RNase	Sigma/Aldrich		Make a 10 mg/ml stock solution.
RNase free 1.5 ml tube	Ambion	AM12450	
RNase Zap	Ambion	AM9780	
Slides 4 wells 11 mm	Thermo Scientific	MENZXER306W	

SSC 20x: NaCl	Sigma/Aldrich	S9625	3 M NaCl (175 g/l) 0.3M Na ₃ citrate x H ₂ O (88 g/l) Adjust to pH 7.0 with 1M HCl
SSC 20x: Sodium citrate dehydrate	Sigma/aldrich	W302600	3 M NaCl (175 g/l) 0.3M Na ₃ citrate x H ₂ O (88 g/l) Adjust to pH 7.0 with 1M HCl
SSPE 20x: NaCl	Sigma/Aldrich	S9625	175.3 g NaCl
SSPE 20x: NaH ₂ PO ₄	Sigma/Aldrich	S0751	27.6 g NaH ₂ PO ₄ .
SSPE 20x: ₄ EDTA powder			9.4 g EDTA powder Add DEPC water, adjust pH 7.4. Autoclave for 20 min
TNB buffer: TNT buffer			10 ml TNT buffer
TNB buffer: Blocking reagent			0.05 g Blocking reagent To dissolve the blocking reagent, heat the solution to 60 °C for one hour with stirring. Store at -20 °C. (Derived from the Perkin Elmer TSA-protocol.)
TNT buffer: Tris/HCl	Sigma	T1503	1M Tris/HCl, pH 8.0
TNT buffer: NaCl	Sigma Aldrich	S9625	100 ml
TNT buffer: Tween20	Sigma	93773	5 M NaCl 30 ml 1 ml DEPC dH ₂ O 869 MI Adjust pH to 7.5 at room temperature. (Derived from the Perkin Elmer TSA-protocol.)
TSA plus Cyanine3/ Fluorescein system	Perkin Elmer	NEL753000IKT	Read the protocol from the TSA Plus Fluorescence Palette System carefully before starting the experiment.
Tubes 14 ml sterile	Almeco - CM LAB Aps	91016	