Materials List for:

Fluorescence Activated Cell Sorting (FACS) and Gene Expression Analysis of Fos-expressing Neurons from Fresh and Frozen Rat Brain Tissue

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Materials

Name	Company	Catalog Number	Comments
Brain matrix	CellPoint Scientific	69-2160-1	to obtain coronal brain slices
Hibernate A low fluorescence	Brain Bits	HA-lf	Buffer A in the protocol is used for processing tissue and cells from the dissociation to fixation steps
Accutase	Millipore	SCR005	Enzyme solution in the protocol is used for enzymatic digestion of tissue prior to trituration
Protein LoBind Tube 1.5 ml, PCR clean	Eppendorf	22431081	to prevent cell lost during the protocol
Cell Strainer, 40 µm	BD Falcon	352340	to filter cell suspension
Cell Strainer, 100 µm	BD Falcon	352360	to filter cell suspension
Falcon 5 ml round-bottom polystyrene test tube with cell strainer snap cap	BD Bioscience	352235	to filter cell suspension before passing though the flow cytometer
Pasteur Pipet, Glass	NIH supply	6640-00-782-6008	to do tissue trituration
Milli-Mark Anti-NeuN-PE, Clone A60 (mouse monoclonal) antibody	EMD Millipore	FCMAB317PE	antibody to detect neurons
Phospho-c-Fos (Ser32) (D82C12) XP Rabbit (Alexa Fluor 647 Conjugate)	Cell Signaling Technology	8677	antibody to detect Fos-expressing cells
DAPI	Sigma	D8417	to label nuclei
PicoPure RNA Isolation Kit	Applied Biosystems.	KIT0204	The kit includes the RNA extraction buffer for step 6.14. It is used to collect sorted cells
Superscript III first strand cDNA synthesis system	Invitrogen	18080-051	to synthesize cDNA from RNA
TaqMan PreAmp Master Mix	Applied Biosystems.	4391128	to do targeted-preamplification from cDNA
TaqMan Advance Fast Master	Applied Biosystems.	4444963	to do PCR using TaqMan probes
Fos TaqMan probe	Applied Biosystems.	Rn00487426_g1	TaqMan probe/primers
NeuN TaqMan probe	Applied Biosystems.	CACTCCAACAGCGTGAC	nucleotide sequence for neuronal gene marker
NeuN Forward primer	Applied Biosystems.	GGCCCCTGGCAGAAAGTAG	nucleotide sequence for neuronal gene marker
NeuN Reverse primer	Applied Biosystems.	TTCCCCCTGGTCCTTCTGA	nucleotide sequence for neuronal gene marker
Gfap TaqMan probe	Applied Biosystems.	Rn00566603_m1	TaqMan probe/primers for astrocityc gene marker

iba-1 TaqMan probe	Applied Biosystems.	Rn00574125_g1	TaqMan probe/primers for microglya gene marker
Gapdh TaqMan probe	Applied Biosystems.	CTCATGACCACAGTCCA	nucleotide sequence for reference/ housekeeping gene
Gapdh Forward primer	Applied Biosystems.	GACAACTTTGGCATCGTGGAA	nucleotide sequence for reference/ housekeeping gene
Gapdh Reverse primer	Applied Biosystems.	CACAGTCTTCTGAGTGGCAGTGA	nucleotide sequence for reference/ housekeeping gene
Oligo2 TaqMan probe	Applied Biosystems.	Rn01767116_m1	TaqMan probe/primers for oligodendrocytic gene marker
P1000 pipettor	Rainin	17014382	It is refered to as the pipette with a large tip diameter in steps 3.1 and 3.3 for mild tissue trituration and step 6.7 to resuspend cells
7500 Fast Real-time PCR system	Applied Biosystems.	446985	for quantitative PCR
FACSAria I Cell Sorter	BD Biosciences		for FACS sorting

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