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Creation of HBSS Wash Buffer | Brusko Lab

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MATERIALS

NAME	CATALOG #	VENDOR
HBSS (1x)	14175-095	Gibco - Thermo Fisher
EDTA (0.5 Molar)	BM-150	Boston Bioproducts
Fetal Bovine Serum (FBS)	25-550	Genesee Scientific
Penicillin/Streptomycin	30-002-CL	Corning

STEPS MATERIALS

NAME	CATALOG #	VENDOR
HBSS (1x)	14175-095	Gibco - Thermo Fisher
Fetal Bovine Serum (FBS)	25-550	Genesee Scientific
Penicillin/Streptomycin	30-002-CL	Corning
EDTA (0.5 Molar)	BM-150	Boston Bioproducts

BEFORE STARTING

Obtain all materials and apparatus required for this procedure. Transfer into a biological safety cabinet using aseptic techniques.

1 Open a sterile 1 liter (L) Rapid-Flow Sterile Disposable Filter Unit with a 0.2 micrometer (µm) PES Membrane.

Rapid-Flow Sterile Disposable Filter Unit with PES Membrane
Filtration System
Nalgene 5670020

2 Remove the lid of the filter flask and add **940 ml** of (1x) HBSS to the filter.

HBSS (1x)
by Gibco - Thermo Fisher
Catalog #: 14175-095

3 Add a single **50 ml** aliquot of FBS and a **10 ml** aliquot of Penicillin/Streptomycin (Pen/Strep) to the HBSS in the filter system

Fetal Bovine Serum (FBS)
by Genesee Scientific
Catalog #: 25-550

Penicillin/Streptomycin
by Corning
Catalog #: 30-002-CL

4 Add **10 µl** of **0.5 Molarity (M)** EDTA to the solution.

EDTA (0.5 Molar)
by Boston Bioproducts
Catalog #: BM-150

5 Process the final solution through the 0.2 µm PES membrane using a positive-pressure system.

6 Once the solution has been filtered, remove the filter from the flask by unscrewing the filter and dispose of the filter into biohazardous waste.

Since this procedure makes use of penicillin and streptomycin, any disposable products that come into contact with these antibiotics should be disposed of in biohazards waste to prevent the creation of antibiotic-resistant bacteria.

7 Remove the flask lid from the secondary container and securely screw the cap onto the flask. Label the flask containing the sterile HBSS Wash Buffer with the following information: "HBSS with FBS, EDTA, Pen/Strep" as well as your name and the date.

8 Store the HBSS Wash Buffer in 4 °C .

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