


STANDARD OPERATING PROCEDURE			
	Sterile Media Preparation		SOP# TC-SOP-015
	Originated by:	Alicja Trocha	Date: 25 December 17
	Reviewed By:	Amruta Samant	Pages: 1 of 2
	Approved by:	Alicja Trocha	

I. PURPOSE

The purpose of this procedure is to establish and outline the requirements for attire and personal protective equipment (PPE) for Ragon Institute laboratories.

II. SCOPE

This procedure applies to all employees, students, contractors and visitors that enter and/or work in the Ragon Institute laboratories.

III. RESPONSIBILITIES

- A. The Ragon Institute **Director and all Laboratory Principal Investigators** are responsible for the overall implementation of this procedure and ensuring compliance.
- B. **Environmental Health & Safety (EH&S)** is responsible for periodically reviewing the application and maintenance of this procedure, and initiating any updates to this procedure.
- C. **All employees, students, contractors and visitors** are required to follow this procedure. Non-compliance with this procedure will result in the assignment of a corrective action plan.
- D. **The Partners Institutional Biosafety Committee, Ragon Laboratory Managers and/or EH&S** are responsible for outlining additional PPE beyond what is stated in Section IV. (Procedure) if warranted through a regulatory requirement or industry best-practice.


IV. PROCEDURE

R-10 (10%FCS)

RPMI 1640 medium (Sigma, St. Louis, Mo)	-500ml bottle
10 % FCS (heat inactivated-1 hour, 56 °C) we buy now HI at the factory	-55ml
L-glutamine (fc 2 mM)	-5ml
Penicillin/streptomycin(FC 50U/ml)	-5ml
Hepes 10mM	-5ml
(N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid)	

R 20 (20% FCS)

RPMI 1640 medium	-500ml
20% FCS (heat inactivated)	-125ml
L-glutamine	-5ml
Penicillin/streptomycin (FC 50U/ml)	-5ml
Hepes 10mM	-6.2ml

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R10/IL-2 medium

Make R10 as outlined above, then add IL-2 at 50U/ml. Use an entire aliquot of IL-2 from the -80° C freezer. The aliquots are made from the Roche stock, which is at 10⁵ U/ml.

Wipe bottles with alcohol after removing them from the water bath. Do not use media directly from the bottle (unless you know you are going to use it up the same day)-- this will give you an extra layer of protection against unexpected contamination. Pour some media into a conical instead and pipet from there. Wipe all bottles with alcohol before putting them in