



SOP-BCR-4.1

**Complete Media Preparation**

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Rev:

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1.0

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Approved: M. Wicha

**1.0 Purpose**

To prepare complete, supplemented tissue culture media.

**2.0 Scope**

This procedure is used to prepare 5% and 10% FBS supplemented media for tissue culture.

**3.0 Description**

Serum and additional supplements are added to tissue culture media prior to use. FBS must be heat inactivated before adding to media to eliminate complement activity. Make sure all reagents are in solution prior to addition to media. Media must be filtered through 0.22um filter prior to use.

**4.0 Materials and Reagents**

No.	Name	Description	Concentration	Storage Location
1.0	Cell Media	RPMI or DMEM	N/A	4°C walk-in (026-380C)
2.0	Penicillin-Streptomycin	10,000 units/ml pen. And 10,000 units/ml strep.	1% (final)	Freezer #2 (026-328S-A)
3.0	L-Glutamine	200mM (100X)	1% (final)	4°C refrig/ -20°C freezer (026-326W-F)
4.0	HEPES	1M	1% (final)	Fridge #5 (026-320S-A)
5.0	Non-essential amino acids	10mM (100X)	1% (final)	4°C refrigerator
6.0	MEM Sodium Pyruvate	100mM	1% (final)	4°C refrigerator
7.0	2-Mercaptoethanol	14.3 M stock	0.05mM (final)	Chemical supply (026-314S)
8.0	FBS	Heat Inactivated Serum	5% or 10% (final)	Freezer #2 (026-328S-A)
9.0	Filter Flasks	Millipore 0.22um filter apparatus	N/A	Supply area

**5.0 Procedure**

5.1 Obtain a 500ml bottle of RPMI or DMEM media from walk-in 4°C cooler (026-380C).

5.2 Add half of the media to a 500ml 0.22um bottle top filter (with the vacuum turned off).

5.3 Add the following:

**5% FBS Media:**

- a. 28ml of FCS
- b. 5.6ml of pen-strep
- c. 5.6ml of L-Glutamine
- d. 5.6ml of HEPES buffer
- e. 5.6ml of Non-essential amino acids
- f. 5.6ml of sodium pyruvate
- g. 0.05ml 2-ME (from 1.0M stock)

**10% FBS Media:**

- a. 59ml of FCS
- b. 6.0ml of pen-strep
- c. 6.0ml of L-Glutamine
- d. 6.0ml of HEPES
- e. 6.0ml of Non-ess. A.A.
- f. 6.0ml of sodium pyruvate
- g. 0.05ml 2-ME

5.4 Apply vacuum to filter system and add the remaining media to the filter.

**6.0 Reference(s)**

Not Applicable.

**7.0 Procedural Change Description**

Rev Level	Date	Reference	Description of Change
1.0	7/13/12	CL	Updated room locations