

## Invasion/migration assay

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### Materials

Cells from 12 well or 6 well ( $1 \times 10^6$  cells are enough for 1x invasion/migration)

24well plate (BD companion plate)

BD BioCoat Growth Factor Reduced MATRIGEL Invasion Chamber

BD control insert (without matrigel)

Accutase

Quick differential staining kit

### Media condition

MCF10A

#### Assay media A (serum free)


<u>Assay media A (serum free)</u>	<u>Amount</u>	<u>Final</u>
DMEM/F12 media	100ml	
BSA (35%)	286ul	0.1%

<u>Assay media B (with chemoattractant)</u>	<u>Amount</u>	<u>Final</u>
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DMEM/F12 media	100ml	
BSA (35%)	286ul	0.1%
Horse serum	5ml	5%
EGF	20ul	20ng/ml

Other cell lines growing in normal DMEM

#### Media A

Serum free (-FBS)

#### Media B

10% FBS

#### Procedure

##### A. Preparation of membrane (Only for inserts with matrigel)

1. Remove the foil package and put at RT until it gets warmed (Don't back to -20oC!)
  2. Rehydrate membranes by media A (500ul for a insert and 500ul for a well)
- (Not to touch the down side of membranes)
3. Incubate at 37oC for 2h (for pH equilibrium)

##### B. Starting assay (30min to 2h)

1. Wash cells with PBS
2. Detach cells by 500ul of accutase, and add 500ul of media A

3. Count cells, spin down, and re-suspend as  $1 \times 10^6/\text{ml}$
4. 200ul of cell suspension and 1.8ml of media A to adjust to  $10 \times 10^4/\text{ml}$  ( $5 \times 10^4/500\text{ul}$ )
5. 750ul of media B in the bottom chamber
6. Remove media from rehydrated inserts and set them into the wells of BD 24 well plate as well as control inserts
7. 500ul of cell mixture in the top chamber
8. Incubate at 37°C 22~24h

#### C. Fixation and staining by Differential Quik Stain Kit

1. Remove media and wash inserts with DW twice
2. Solution A for 1min followed by DW wash twice
3. Solution B for 1min followed by DW wash twice
4. Solution C for 1min followed by DW wash twice
5. Remove cells on upside by scrubbing with cotton buds
6. DW wash twice
7. Dry up inserts
8. Observe by microscope and count, if necessary, cut membranes and put on slide glasses