# NGFR-mNG2 sorting protocol

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**Abstract**

Protocol for preparing NGFR-mNG2 tagged WM989 A6-G3 melanoma cells for sorting.

**Citation:** Benjamin Emert NGFR-mNG2 sorting protocol. **protocols.io** dx.doi.org/10.17504/protocols.io.rh6d39e
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## Before start

Warm trypsin, media and DPBS.

## Materials

7-AAD Viability Staining Solution 420403 by BioLegend

12x75 mm high clarity polypropylene test tubes 352063 by Corning

Falcon® 5 mL Round Bottom Polystyrene Test Tube, with Cell Strainer Snap Cap 352235 by Corning

DPBS no calcium, no magnesium 14190136 by Invitrogen - Thermo Fisher

0.05% Trypsin-EDTA, phenol red 25300054 by Invitrogen - Thermo Fisher

Propidium iodide staining solution 556463 by BD Biosciences

DAPI D3571 by Invitrogen - Thermo Fisher

UltrapPure 0.5M EDTA pH 8.0 15575020 by

Invitrogen - Thermo Fisher

TU2% View by Contributed by users

15mLl polypropylene centrifuge tubes
352096 by Corning

## Protocol

### Trypsinize and pellet cells

***Step 1.***

Wash 5 confluent 10cm plates with 1xDPBS

NOTES

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For the NGFR-mNG2 WM989 A6-G3 cell lines you can expect 4-5 million cells on a confluent 10cm dish. Scale up the number of starting plates accordingly.

### Trypsinize and pellet cells

***Step 2.***

Aspirate 1xDPBS and add 2mL 0.05% Trypsin-EDTA. Incubate at 37ºC for 3-5 minutes.

AMOUNT

2 ml Additional info:
0.05% Trypsin EDTA

TEMPERATURE

37 °C Additional info:
incubate

### Trypsinize and pellet cells

***Step 3.***

Neutralize trypsin with 4mL TU2% media. Transfer dissociated cells to 15mL falcon tube.

AMOUNT

4 ml Additional info: TU2%
media

NOTES

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Can use 1 15mL falcon tube for 2.5 10cm plates. Or scale down the volume of trypsin and media to fit more cells into fewer tubes.

### Trypsinize and pellet cells

***Step 4.***

Pellet cells by centrifugation at 1,000 rpm (200rcf) for 3 minutes.

NOTES

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During centrifugation, prepare sterile 1xDPBS with 2mM EDTA. Prepare excess.

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Alternatively, can centrifuge at 300 rcf

## Wash cell pellets

***Step 5.***

Resuspend cell pellet in 5mL 1xDPBS with 2mM EDTA.

AMOUNT

5 ml Additional info:
1xDPBS with 2mM EDTA

NOTES

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Can combine pellets at this point

## Wash cell pellets

***Step 6.***

Pellet cells by centrifugation at 1,000 rpm (200rcf) for 3 minutes.

NOTES

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During centrifugation prepare 1xDPBS with 2mM EDTA and live dead staining solution. I use one of the following:
4uL/mL 7-AAD (final concentration 200ng/mL)
2uL/mL Propidium iodide (final concentration 100ng/mL)
1uL/mL DAPI (final concentration 50ng/mL).

Prepare excess for diluting cells if necessary.

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During centrifugation prepare sterile 5mL polypropylene FACS tubes with 1mL TU2% for collecting sorted cells.

## Stain dead cells

***Step 7.***

Resuspend cell pellets in 1xDPBS with 2mM EDTA and dead cell stain. I will typically use 6mL for 5-10cm plate's worth of cells.

## Filter cells

***Step 8.***

Remove cell clumps by passing cell suspension through cell strainer cap into sterile 5mL polypropylene

FACS tubes. Place tubes on ice.

## Proceed with FACS

***Step 9.***

Bring with you the following:

- 1xDPBS with 2mM EDTA and dead cell stain for diluting cells
- 5mL polypropylene FACS tubes with 1mL TU2% for collecting cells
- Excess FACS tubes and media