# How to use these sheets

One sheet per unit operation, ten in all, covering the 21 canonical CP-2 steps (buffer prep, D0.1-D0.5, Days 1-4, D4.1-D4.15). The left half restates the step as RTx described it (clock, grade, equipment, in-process tests, the change vs CP-1). The right half is ruled space for what the room decides. Write in pen; the sheets are the workshop record and are transcribed into the action log within 24 h.

Clocks: 'RTx stated' figures come from the RTx 'US CDMO - Pivotal Process Detailed' docx (3-4 Sep 2026) and briefing pack slides 4-9. 'MADE working estimate' figures come from the MADE capacity model and are to be confirmed at the Day-4 rehearsal; they are not facts. Day 4 elapsed time: the RTx PFD implies ~20 h harvest-to-CRF; the MADE working estimate is 12 h (target 11.5-14 h).

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| 2 | **Monocyte seeding into MACS1000 bags via Prodigy formulation** | Day 0 | RTx 1 h (TBC) | *MADE n/a* | C1, C2, C3 |
| 3 | **Monocyte-to-macrophage differentiation** | Days 1-4 | RTx 96 h | *MADE n/a* | C4 |
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| 5 | **Transfection preparation and Flowfect electroporation** | Day 4 | RTx 1.67 h | *MADE 2.25 h* | C5, C9 |
| 6 | **Recovery culture** | Day 4 | RTx 5.17 h (TBC) | *MADE 5.17 h* | C6 |
| 7 | **Harvest, Rotea wash into saline and dose setting** | Day 4 into 5 | RTx 2.83 h | *MADE 3.75 h* | none |
| 8 | **DS and FDP formulation and fill on Ares X20** | Day 4 into 5 | RTx 3.17 h (TBC) | *MADE 1.92 h* | C1, C7 |
| 9 | **Visual inspection, cassette, controlled-rate freeze and VPLN2** | Day 4 into 5 | RTx 0.58 h | *MADE 1.75 h* | C8 |


## The eight changes (C1-C8) and the mRNA supply variable (C9)

C1 · Liquid handling: Fresenius Cue for fluid transfer, formulation and fill to Cue replaced (Prodigy formulation bar at D0; Ares X20 at fill). RTx rationale: More appropriate options available; replacements being tested; data pending.

C2 · Culture bag fill: CellConnect / manual fill to CliniMACS Prodigy formulation bar. RTx rationale: Closed fill from Prodigy.

C3 · Culture bag size: up to 18-20 MACS500 to up to 10 MACS1000. RTx rationale: Reduce bag count and handling.

C4 · Macrophage conversion period: 5 days to 4 days. RTx rationale: Plateau in differentiation markers; reduced facility time.

C5 · Electroporation: Lonza 4D-Nucleofector LV to Kytopen Flowfect (TBC). RTx rationale: Faster throughput, qualification, customization.

C6 · Post-EP recovery / time to cryo: Overnight on magnetic stirrer (D5-D6) to ~4 h (2-6 h window) TBC. RTx rationale: Optimizing mRNA quantity in DP; IL-10 OOS risk at lower limit; capitalize on expression before mRNA decays.

C7 · Formulation / fill automation: Cue or manual to Xiogenix Ares X20. RTx rationale: Reduce manual handling, increase sterility assurance.

C8 · Cryopreservation: Planer CRF, current cassette to TF CryoMed LN2 CRF (or other) + metal cassette. RTx rationale: CDMO availability and broader access for pivotal.

C9 · mRNA supply (not a process change but a comparability variable): MS-1 (EU provider) to MS-2 (new provider). RTx rationale: Commercial-appropriate supply.

Sources: ruby_process.json v1.0 (MADE, 26 Sep 2026); RTx 'US CDMO - Pivotal Process Detailed' docx (S. Mellon / S. Howe, 3-4 Sep 2026); RTx Workshop Briefing Pack vFinal slides 4-9 (4 Sep 2026); MADE capacity model working estimates (Sep 2026); RTx x MADE pre-briefing (21 Sep 2026). US English. Equipment named is the CP-2 process equipment as stated by RTx.

## 0 Buffer and reagent preparation

A (ISO 5) | Day -15 to -1

**Clock**
**RTx stated: 3 h**

**Steps (RTx docx)**
PREP · Process buffer, DS formulation buffer, electroporation buffer, base culture medium, recovery medium · 3 h

**Starting material and inputs**
· Raw materials (buffers, media, reagents)
· RTX001_01 mRNA vial thawed and transferred to a weldable storage pouch (Grade A; new in CP-2)

**In-process test / sample**
*no IPC at this unit operation*

**Key equipment and consumable**
Peristaltic pump; Balance; Tube welder; Heat sealer; Class A MSC

**The change vs CP-1**
*no numbered change at this unit operation*

**Note**
Buffers and media are prepared ahead of the run in a Grade A environment. New in CP-2: the mRNA vial is thawed and transferred to a weldable storage pouch so it can be welded into the closed Day 4 transfection set-up.

### RTx change and data status

### MADE view

### Optimization ideas

### Operational impact
facility / equipment / staffing / QC

*facility*

*equipment*

*staffing*

*QC*

### Data needed

### Decision

### Owner

### Date

## 1 Starting-material assessment and CD14+ enrichment

C (ISO 7) | Day 0

**Clock**

**RTx stated: 10.83 h**

**Steps (RTx docx)**

D0.1 · Equipment set-up · 0.5 h
D0.2 · Cellular starting-material assessment · 0.5 h
D0.3 · Pre-enrichment prep (loading, priming) · 3.33 h
D0.4 · CD14+ enrichment run (selection + wash) · 6.5 h

**Starting material and inputs**

· Leukapheresis cell material: receipt within 48 h of collection; apheresis Tue/Wed (pivotal), arrival next afternoon; Day 0 start within 24 h of receipt

**In-process test / sample**

· CD45+ count (NC-202)
· %CD14+ (BD FACSLyric)
· CD45+ count post-enrichment

**Key equipment and consumable**

CliniMACS Prodigy (Miltenyi); NC-202 (ChemoMetec); BD FACSLyric; Class A MSC

**The change vs CP-1**

*no numbered change at this unit operation*

**Note**

Prodigy CD14 input envelope (MATCH precedent): up to 20e9 WBC, under 400e6 WBC/mL, up to 3.5e9 CD14+, 50-300 mL. Starting-material assessment sets the CD45+ count and %CD14+ before enrichment.

### RTx change and data status

### MADE view

### Optimization ideas

### Operational impact

facility / equipment / staffing / QC

*facility*

*equipment*

*staffing*

*QC*

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### Decision

### Owner

### Date

## 2 Monocyte seeding into MACS1000 bags via Prodigy formulation bar

C (ISO 7) | Day 0

**Clock**

**RTx stated: 1 h (TBC)**

**Steps (RTx docx)**

D0.5 · Monocyte seeding into up to 10 MACS1000 differentiation bags via Prodigy formulation bar · 1 h TBC

**Starting material and inputs**

· Up to 10 x MACS1000 differentiation bags (replaces up to 18-20 x MACS500)

**In-process test / sample**

· Cell count end of Day 0 (NC-202)

**Key equipment and consumable**

Prodigy formulation bar (custom application from Miltenyi); MACS1000 bags; CO2 incubator

**The change vs CP-1**

**C1 Liquid handling: Fresenius Cue for fluid transfer, formulation and fill to Cue replaced (Prodigy formulation bar at D0; Ares X20 at fill)**

RTx rationale: More appropriate options available; replacements being tested; data pending. RTx asks: CDMO experience of equipment?

**C2 Culture bag fill: CellConnect / manual fill to CliniMACS Prodigy formulation bar**

RTx rationale: Closed fill from Prodigy. RTx strategy: Updated CAP required, timelines, limited bag number, filtered air required. RTx asks: CDMO input: space / Prodigy usage

**C3 Culture bag size: up to 18-20 MACS500 to up to 10 MACS1000**

RTx rationale: Reduce bag count and handling. RTx strategy: Checking CQA impact (no issues so far). RTx asks: Incubator / manual handling preference / facility fit

**Note**

Replaces up to 18-20 MACS500 bags filled via Cue/CellConnect. A custom application (CAP) is required from Miltenyi for the formulation bar; filtered air is required. Bags go to the CO2 incubator.

### RTx change and data status

### MADE view

### Optimization ideas

### Operational impact

facility / equipment / staffing / QC

*facility*

*equipment*

*staffing*

*QC*

### Data needed

### Decision

### Owner

### Date

## 3 Monocyte-to-macrophage differentiation

C (ISO 7) · Days 1-4

**Clock**
**RTx stated: 96 h**

**Steps (RTx docx)**
D1-4 · 4-day culture in serum-free medium + M-CSF (was 5 days in CP-1) · 96 h

**Starting material and inputs**
· Serum-free medium + M-CSF (MATCH precedent: TexMACS + 100 ng/mL M-CSF, 50% feeds d2/d4). Feed schedule for the 4-day culture is not stated in the CP-2 docx: to confirm

**In-process test / sample**
*no IPC at this unit operation*

**Key equipment and consumable**
CO2 incubator (bags per shelf and incubators per batch: open); MACS1000 differentiation bags

**The change vs CP-1**
C4 **Macrophage conversion period: 5 days to 4 days**
RTx rationale: Plateau in differentiation markers; reduced facility time. RTx strategy: Ongoing CQA / molecular pathway (omics) data. RTx asks: Throughput and starts

**Note**
4 days (was 5). RTx rationale: plateau in differentiation markers and reduced facility time; CQA and omics data ongoing. Incubator fit (bags per shelf, incubators per batch) is open and drives the facility model.

### RTx change and data status

### MADE view

### Optimization ideas

### Operational impact
facility / equipment / staffing / QC

*facility*

*equipment*

*staffing*

*QC*

### Data needed

### Decision

### Owner

### Date

## 4 Harvest, pooling and Rotea wash into electroporation buffer

C (ISO 7) | Day 4

**Clock**

**RTx stated: 7.33 h**

*MADE working estimate: 3.33 h (rehearsal to confirm)*

**Steps (RTx docx)**

D4.1 · Harvest set-up · 0.33 h

D4.2 · Macrophage harvest and pooling (up to 10 bags) · 2.75 h
*MADE 1.75 h (working estimate)*

D4.3 · Rotea wash and buffer exchange into electroporation buffer · 4.25 h
*MADE 1.25 h (working estimate)*

**Starting material and inputs**

· Wash buffer; electroporation buffer

**In-process test / sample**

· Cell count start of Day 4 (NC-202)
· Cell count after buffer exchange

**Key equipment and consumable**

BSC / bench; Tube welder; Rotea (Thermo Fisher); NC-202

**The change vs CP-1**

*no numbered change at this unit operation*

**Note**

RTx comment on D4.2: the 7 h harvest-plus-wash figure is MACS500 time and is expected to be less with MACS1000. RTx comment on D4.3: the wide range likely reflects variable cell loads.

### RTx change and data status

### MADE view

### Optimization ideas

### Operational impact

facility / equipment / staffing / QC

*facility*

*equipment*

*staffing*

*QC*

### Data needed

### Decision

### Owner

### Date

## 5 Transfection preparation and Flowfect electroporation

C (ISO 7) · Day 4

**Clock**

**RTx stated: 1.67 h**

*MADE working estimate: 2.25 h (rehearsal to confirm)*

**Steps (RTx docx)**

D4.4 · Transfection set-up and cell/mRNA/buffer preparation · 1.5 h

D4.5 · Electroporation (Kytopen Flowfect, continuous flow) · 0.17 h

*MADE 0.75 h (working estimate)*

**Starting material and inputs**

· mRNA RTX001_01 (RTx-supplied). MS-1 to MS-2 supply change is a comparability variable (C9)
· Electroporation buffer

**In-process test / sample**

· Cell count before transfection
· Post-transfection cell count

**Key equipment and consumable**

Balance; Tube welder; NC-202; Kytopen Flowfect electroporator (continuous flow)

**The change vs CP-1**

C5 **Electroporation: Lonza 4D-Nucleofector LV to Kytopen Flowfect (TBC)**

RTx rationale: Faster throughput, qualification, customization. RTx strategy: Equipment being tested; CDMO quality interactions. RTx asks: Any concerns?

C9 **mRNA supply (not a process change but a comparability variable): MS-1 (EU provider) to MS-2 (new provider)**

RTx rationale: Commercial-appropriate supply. RTx strategy: Small-scale comparison underway.

**Note**

Adjust to target concentration (patent: 50-100e6/mL small scale, 200e6/mL large-scale fluidics), load the cartridge and calculate the mRNA volume for the mRNA:cell ratio. Flowfect run ~5 min plus ~5 min close-out; RTx total transfection block 1.75 h. Replaces the Lonza 4D-Nucleofector LV.

### RTx change and data status

### MADE view

### Optimization ideas

### Operational impact

facility / equipment / staffing / QC

*facility*

*equipment*

*staffing*

*QC*

### Data needed

### Decision

### Owner

### Date

## 6 Recovery culture

C (ISO 7) Day 4

**Clock**

**RTx stated: 5.17 h (TBC)**

*MADE working estimate: 5.17 h (rehearsal to confirm)*

**Steps (RTx docx)**

D4.6 · Recovery medium addition and transfer to up to 4 MACS bags · 1.17 h

D4.7 · Recovery culture · 4 h TBC

*MADE 4 h (working estimate)*

**Starting material and inputs**

· Recovery medium; up to 4 MACS bags (how many and which size: open)

**In-process test / sample**

*no IPC at this unit operation*

**Key equipment and consumable**

Tube welder; Balance; CO2 incubator

**The change vs CP-1**

C6 **Post-EP recovery / time to cryo: Overnight on magnetic stirrer (D5-D6) to ~4 h (2-6 h window) TBC**

RTx rationale: Optimizing mRNA quantity in DP; IL-10 OOS risk at lower limit; capitalize on expression before mRNA decays. RTx strategy: CQA data collection. RTx asks: Batch throughput and staffing; does it fit CDMO shifts?

**Note**

RTx rationale for the ~4 h recovery (2-6 h window, TBC): mRNA residency decays (~3% of copies at 16 h) while IL-10 secretion is cumulative to ~16 h; use expression before decay and reduce OOS risk at the IL-10 lower limit. Bags returned to the incubator (how many, which size) is open.

### RTx change and data status

### MADE view

### Optimization ideas

### Operational impact

facility / equipment / staffing / QC

*facility*

*equipment*

*staffing*

*QC*

### Data needed

### Decision

### Owner

### Date

## 7 Harvest, Rotea wash into saline and dose setting

C (ISO 7/8) | Day 4 into 5

**Clock**

**RTx stated: 2.83 h**

*MADE working estimate: 3.75 h (rehearsal to confirm)*

**Steps (RTx docx)**

D4.8 · Harvest set-up · 0.5 h

D4.9 · Harvest and pooling into harvest bag · 1 h

D4.10 · Rotea wash and concentrate into saline (intermediate bag) · 0.33 h

*MADE 1.25 h (working estimate)*

D4.11 · Post-Rotea to loading on Ares X20 (waiting for stained TruCount) · 1 h

**Starting material and inputs**

· Saline (intermediate bag)

**In-process test / sample**

· Post-harvest count

· Post-Rotea count

· TruCount viable macrophage enumeration (BD FACSLyric): gates the fill

**Key equipment and consumable**

BSC / bench; Rotea (Thermo Fisher); NC-202; BD FACSLyric

**The change vs CP-1**

*no numbered change at this unit operation*

**Note**

Dose setting is performed on drug substance: the stained TruCount result gates the fill. D4.11 is the wait for TruCount between Rotea and loading on the Ares X20.

### RTx change and data status

### MADE view

### Optimization ideas

### Operational impact

facility / equipment / staffing / QC

*facility*

*equipment*

*staffing*

*QC*

### Data needed

### Decision

### Owner

### Date

## 8 DS and FDP formulation and fill on Ares X20

C (ISO 7/8) | Day 4 into 5

**Clock**

**RTx stated: 3.17 h (TBC)**

*MADE working estimate: 1.92 h (rehearsal to confirm)*

**Steps (RTx docx)**

D4.12 · DS formulation (dilute in DS formulation buffer) then FDP formulation (CryoStor CS10 addition) · 2.5 h TBC
*MADE 1.25 h (working estimate)*

D4.13 · Container prep and Ares set-up; bag fill (bags 1-20); QC vial fill · 0.67 h

**Starting material and inputs**

· DS formulation buffer; CryoStor CS10
· CryoMACS 50 bags (10 mL fill at 1e7 cells/mL; 4-9 bags per RTx slide 6, up to 20 fill positions); ~20 x 1 mL QC vials

**In-process test / sample**

· QC samples for release and characterization taken here (see panel)
· Cell count results

**Key equipment and consumable**

Ares X20 (Xiogenix); Heat sealer; Balance

**The change vs CP-1**

C1 **Liquid handling: Fresenius Cue for fluid transfer, formulation and fill to Cue replaced (Prodigy formulation bar at D0; Ares X20 at fill)**

RTx rationale: More appropriate options available; replacements being tested; data pending. RTx asks: CDMO experience of equipment?

C7 **Formulation / fill automation: Cue or manual to Xiogenix Ares X20**

RTx rationale: Reduce manual handling, increase sterility assurance. RTx strategy: Water/buffer runs complete, cell tests underway. RTx asks: CDMO view on equipment and space

**Note**

DP: CryoMACS 50 bags, 10 mL fill at 1e7 cells/mL, multiple bags (4-9 per RTx slide 6, up to 20 fill positions) plus ~20 x 1 mL QC vials. DP bag size drives LN2 sizing (open). RTx docx asks whether visual inspection can run concurrently.

### RTx change and data status

### MADE view

### Optimization ideas

### Operational impact

facility / equipment / staffing / QC

*facility*

*equipment*

*staffing*

*QC*

### Data needed

### Decision

### Owner

### Date

## 9 Visual inspection, cassette, controlled-rate freeze and VPLN2

C (ISO 7/8) | Day 4 into 5

**Clock**

**RTx stated: 0.58 h**

*MADE working estimate: 1.75 h (rehearsal to confirm)*

**Steps (RTx docx)**

D4.14 · Post-fill visual inspection, label, over-wrap, vacuum seal, metal cassette, temporary fridge · 0.25 h
*MADE 0.75 h (working estimate)*

D4.15 · MSC close and transfer to controlled-rate freezer; CRF run; transfer to VPLN2 · 0.33 h
*MADE 1 h (working estimate)*

**Starting material and inputs**

· Labels, over-wrap, metal cassette

**In-process test / sample**

· Visual inspection USP <790>

**Key equipment and consumable**

VI light box; Vacuum sealer; Temporary fridge; Controlled-rate freezer (TF CryoMed LN2; Planer historically); VPLN2 storage

**The change vs CP-1**

C8 **Cryopreservation: Planer CRF, current cassette to TF CryoMed LN2 CRF (or other) + metal cassette**

RTx rationale: CDMO availability and broader access for pivotal. RTx strategy: Temperature mapping / equipment suitability not yet initiated; potential CDMO work package. RTx asks: How would you approach CRF mapping and validation?

**Note**

CRF mapping and validation not yet initiated by RTx; cassette change pending. Three-way matrix to bridge: ViaFreeze (RTx lab), LN2 CRF (EMERALD) and CryoMed (MADE).

### RTx change and data status

### MADE view

### Optimization ideas

### Operational impact

facility / equipment / staffing / QC

*facility*

*equipment*

*staffing*

*QC*

### Data needed

### Decision

### Owner

### Date