LAMPIRAN

Lampiran 1. Alat-alat yang Digunakan dalam Penelitian

Oven Froilabo

Cooling rack

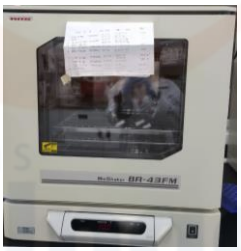
Autoklaf Hirayama

Kapas dan kasa

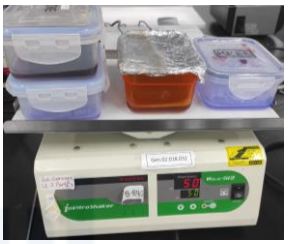
Freezer Thermo Scientific

Kulkas Biobase dan Polytron

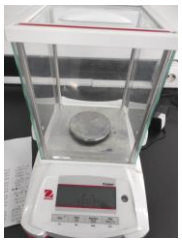
SDS-PAGE Bio-rad



Shaker Bioshaker



Digital shaker *circular rocker* Invitroshaker



Timbangan analitik Ohaus (miligram)

Timbangan analitik Fujitsu (gram)

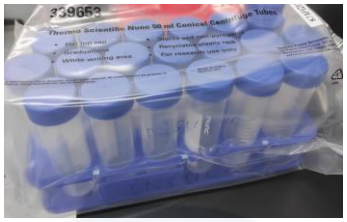
Laminar air flow Thermo Scientific

Cryotube Corning

pH meter

Tisu Hytech untuk membersihkan kuvet

Microtips set (10 µL, 100 µL, 1000 µL)



Centrifuge tube Thermo Scientific

Micro tube

Sentrifugasi Thermo Scientific

Spin down centrifuge Dlab

Digital heating shaking drybath Thermo Scientific

Hot plate magic stirer Heidolph

Spuit dan *syringe filter* Sartorius Stedim

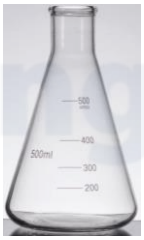
Mikropipet (0,5-10 µL, 0,2-20 µL, 20-200 µL, 100-1000 µL)

Gloves Sensi

Kuvet

Spektrofotometer Shimadzu

Destruk Tomy



Erlenmeyer flask Iwaki

Baffled flask Duran

Wadah pewarnaan gel

Alumunium foil

Vortex Starlab

Botol laboratorium Duran

Spatula

Microtube rack dan *Detachable centrifuge tube*

Beaker glass Iwaki

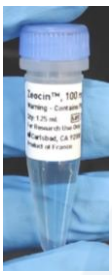
Mikrosentrifus Thermo Scientific

Lampiran 2. Bahan yang Digunakan dalam Penelitian

Pichia pastoris HF7 yang membawa kaset ekspresi pD902-IP-*full-length α-factor*

Media YPD (*Yeast extract, peptone, d-glucose*) cair

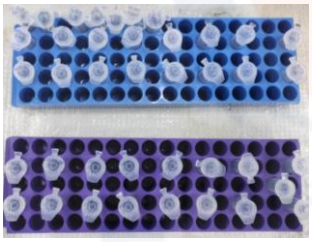
Aquadest steril



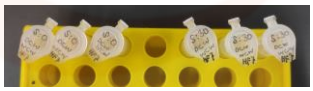
Zeocin™ 100 µg/mL Invitrogen

Human insulin-USP Sigma-Aldrich

Precision plus protein dual xtra standard Bio-rad



Sampel protein prekursor *human* insulin klon HF7 dan WT batch 1 dan batch 2



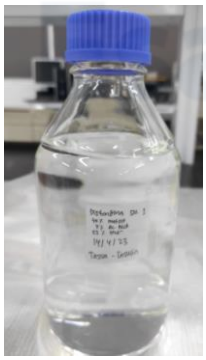
Sampel protein prekursor *human* insulin klon HF7 batch 3

Preparasi larutan untuk pembuatan gel acrylamid 15%

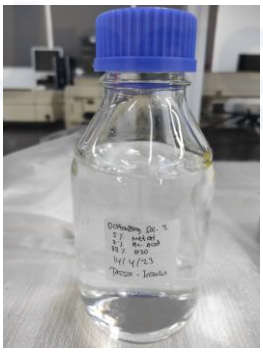
Tri-MOPS-SDS running *buffer* powder GenScript

Gel precast 4-20% GenScript

Buffer MOPS



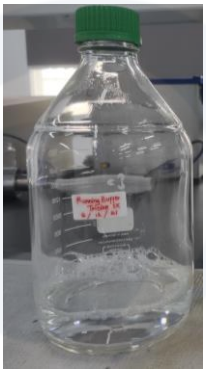
Distanding *solution* 1



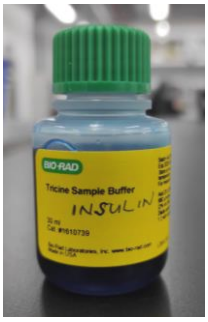
Distanding *solution 2*

Media BSMM 3% (*Methanol basal salt medium*)

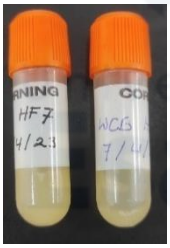
Pewarna CBB (*Coommassie Briliant Blue*) Bio-Rad



Buffer tricine

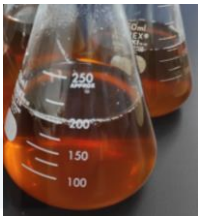


Pelarut sampel protein batch 1, batch 2, dan batch 3

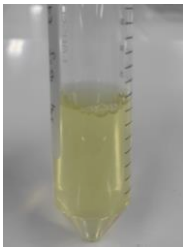


WCB HF7 dan WCB WT (*Wild Type*)

Alkohol 70%



Media BMGY (*Buffered glycerol complex medium*)



Induksi HF7

Resolving gel 15%

Stacking gel 4%

Lampiran 3. Perhitungan Penambahan Sorbitol pada Awal Ekspresi

1. 10 g/L dalam 25 mL $\longrightarrow$ $\frac{25\ mL}{1000\ mL}\ x\ 10\ g = 0{,}25\ g$
2. 20 g/L dalam 25 mL $\longrightarrow$ $\frac{25\ mL}{1000\ mL}\ x\ 20\ g = 0{,}5\ g$
3. 30 g/L dalam 25 mL $\longrightarrow$ $\frac{25\ mL}{1000\ mL}\ x\ 30\ g = 0{,}75\ g$
4. 40 g/L dalam 25 mL $\longrightarrow$ $\frac{25\ mL}{1000\ mL}\ x\ 40\ g = 1\ g$
5. 50 g/L dalam 25 mL $\longrightarrow$ $\frac{25\ mL}{1000\ mL}\ x\ 50\ g = 1{,}25\ g$
6. 60 g/L dalam 25 mL $\longrightarrow$ $\frac{25\ mL}{1000\ mL}\ x\ 60\ g = 1{,}5\ g$

Lampiran 4. Data Nilai OD_{600} Batch 1, Batch 2, dan Batch 3

Nilai OD_{600} *Pichia pastoris* klon HF7 dan WT pada 0 Jam dan 72 Jam (*baffled flask* dan *erlenmeyer flask*) batch 1

Jenis *Flask*	Perlakuan Sorbitol (g/L)	Nilai OD_{600} (0 Jam)	Nilai OD_{600} (72 Jam)
Baffled Flask	HF7 S0	18,66	23,33
	HF7 S10	17,68	23,79
	HF7 S20	17,87	23,90
	HF7 S30	16,98	24,31
	HF7 S40	17,77	24,15
	HF7 S50	17,77	24,10
	HF7 S60	17,45	24,02
	WT	15,41	22,13
Erlenmeyer flask	HF7 S0	17,73	22,21
	HF7 S10	17,89	23,24
	HF7 S20	17,66	25,03
	HF7 S30	17,27	24,85
	HF7 S40	17,42	24,79
	HF7 S50	17,80	24,47
	HF7 S60	17,80	24,71
	WT	14,47	20,55

Nilai OD_{600} *Pichia pastoris* klon HF7 pada 0 Jam dan 96 Jam (*baffled flask* dan *erlenmeyer flask*) batch 2

Jenis *Flask*	Perlakuan Sorbitol (g/L)	Nilai OD_{600} (0 Jam)	Nilai OD_{600} (96 Jam)
Baffled Flask	HF7 S0	17,85	23,16
	HF7 S10	18,20	23,82
	HF7 S20	18,47	24,60
	HF7 S30	19,24	25,57
	HF7 S40	17,96	24,77
	HF7 S50	18,23	25,58
	HF7 S60	18,77	25,59
Erlenmeyer Flask	HF7 S0	17,08	21,66
	HF7 S10	18,49	23,57
	HF7 S20	18,08	23,11
	HF7 S30	17,25	22,94
	HF7 S40	18,37	23,77
	HF7 S50	16,98	22,90

Jenis *Flask*	Perlakuan Sorbitol (g/L)	Nilai OD_{600} (0 Jam)	Nilai OD_{600} (96 Jam)
	HF7 S60	17,54	24,06

Nilai OD_{600} *Pichia pastoris* klon HF7 pada S0 dan S30 (*erlenmeyer flask*) batch 3

Jenis *Flask*	Perlakuan Sorbitol (g/L)	Nilai OD_{600} (0 Jam)	Nilai OD_{600} (96 Jam)	Selisih Nilai OD_{600} Pasca 96 Jam
Erlenmeyer Flask	HF7 S0	20,84	25,64	4,80
	HF7 S0	21,46	25,87	4,41
	HF7 S0	21,01	25,99	4,98
	HF7 S30	20,92	26,00	5,08
	HF7 S30	20,67	26,62	5,95
	HF7 S30	20,61	26,70	6,09

Lampiran 5. Analisis Statistik Nilai OD600 Batch 3

Tests of Normality

	Kadar_Sorbitol	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
Selisih_Nilai_OD600	S0	,262	3	.	,957	3	,600
	S30	,338	3	.	,852	3	,245

a. Lilliefors Significance Correction

Hasil uji normalitas nilai OD_{600} Batch 3

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
Selisih_Nilai_OD600	Equal variances assumed	2,311	,203	-2,729	4	,026	,053	-,97667	,35793	-1,97043	,01710
	Equal variances not assumed			-2,729	3,050	,035	,071	-,97667	,35793	-2,10531	,15197

Hasil uji homogenitas dan uji T-Test nilai OD_{600} batch 3

Lampiran 6. Data Penimbangan WCW/DCW Batch 1, Batch 2, Batch 3

Berat sel basah (WCW) *Pichia pastoris* klon HF7 dan WT pada 0 jam dan 72 jam (*baffled flask* dan *erlenmeyer flask*) batch 1

Jenis *Flask*	Perlakuan Sorbitol (g/L)	Sel Basah (0 Jam) (g)	Sel Basah (72 Jam) (g)
Baffled Flask	HF7 S0	1,950	3,138
	HF7 S10	1,655	3,110
	HF7 S20	1,840	3,754
	HF7 S30	1,860	3,810
	HF7 S40	1,545	3,411
	HF7 S50	1,943	3,591
	HF7 S60	1,718	3,397
	WT	1,595	2,564
Erlenmeyer Flask	HF7 S0	1,523	2,794
	HF7 S10	1,888	3,614
	HF7 S20	1,685	3,365
	HF7 S30	1,603	3,116
	HF7 S40	1,500	3,084
	HF7 S50	1,670	3,205
	HF7 S60	1,728	3,404
	WT	2,485	3,580

Berat sel kering (DCW) *Pichia pastoris* klon HF7 dan WT pada 0 jam dan 72 jam (*baffled flask* dan *erlenmeyer flask*) batch 1

Jenis *Flask*	Perlakuan Sorbitol (g/L)	Sel Kering (0 Jam) (g)	Sel Kering (72 Jam) (g)
Baffled Flask	HF7 S0	0,332	0,652
	HF7 S10	0,300	0,720
	HF7 S20	0,312	0,909
	HF7 S30	0,292	0,870
	HF7 S40	0,290	0,896
	HF7 S50	0,315	0,893
	HF7 S60	0,300	0,882
	WT	0,115	0,350
Erlenmeyer Flask	HF7 S0	0,295	0,646
	HF7 S10	0,338	0,809
	HF7 S20	0,303	0,785

Jenis *Flask*	Perlakuan Sorbitol (g/L)	Sel Kering (0 Jam) (g)	Sel Kering (72 Jam) (g)
	HF7 S30	0,303	0,782
	HF7 S40	0,303	0,778
	HF7 S50	0,330	0,814
	HF7 S60	0,345	0,904
	WT	0,225	0,508

Berat sel basah (WCW) *Pichia pastoris* klon HF7
pada 0 jam dan 96 jam (*baffled flask* dan *erlenmeyer flask*) batch 2

Jenis *Flask*	Perlakuan Sorbitol (g/L)	Sel Basah (0 Jam) (g)	Sel Basah (96 Jam) (g)
Baffled Flask	HF7 S0	2,340	2,420
	HF7 S10	2,173	2,261
	HF7 S20	2,538	2,652
	HF7 S30	2,400	2,520
	HF7 S40	2,063	2,189
	HF7 S50	2,510	2,637
	HF7 S60	2,600	2,716
Erlenmeyer Flask	HF7 S0	2,000	2,082
	HF7 S10	2,048	2,153
	HF7 S20	2,133	2,241
	HF7 S30	2,208	2,312
	HF7 S40	2,665	2,768
	HF7 S50	2,283	2,366
	HF7 S60	1,998	2,097

Berat sel kering (DCW) *Pichia pastoris* klon HF7
pada 0 jam dan 96 jam (*baffled flask* dan *erlenmeyer flask*) batch 2

Jenis *Flask*	Perlakuan Sorbitol (g/L)	Sel Kering (0 Jam) (g)	Sel Kering (96 Jam) (g)
Baffled Flask	HF7 S0	0,462	0,482
	HF7 S10	0,543	0,565
	HF7 S20	0,745	0,772
	HF7 S30	0,622	0,652
	HF7 S40	0,515	0,546
	HF7 S50	0,870	0,901

Jenis *Flask*	Perlakuan Sorbitol (g/L)	Sel Kering (0 Jam) (g)	Sel Kering (96 Jam) (g)
	HF7 S60	0,677	0,708
Erlenmeyer Flask	HF7 S0	0,467	0,485
	HF7 S10	0,582	0,606
	HF7 S20	0,580	0,606
	HF7 S30	0,438	0,464
	HF7 S40	0,630	0,655
	HF7 S50	0,755	0,776
	HF7 S60	0,570	0,597

Berat sel basah (WCW) dan sel kering (DCW) *Pichia pastoris* klon HF7 pada 0 jam dan 96 jam (*erlenmeyer flask*) batch 3

Jenis *Flask*	Jenis Sel	Perlakuan Sorbitol (g/L)	Waktu (0 Jam) (g)	Waktu (96 Jam) (g)	Penambahan berat sel (96 jam) (g)
Erlenmeyer Flask	Sel Basah	HF7 S0	4,425	7,590	3,165
		HF7 S0	4,348	7,432	3,084
		HF7 S0	4,948	7,921	2,973
		HF7 S30	3,918	7,174	3,256
		HF7 S30	3,828	7,428	3,600
		HF7 S30	3,940	7,293	3,353
	Sel Kering	HF7 S0	1,030	1,832	0,802
		HF7 S0	0,960	1,765	0,805
		HF7 S0	1,045	1,830	0,785
		HF7 S30	0,992	1,992	1,000
		HF7 S30	0,905	1,971	1,066
		HF7 S30	0,955	1,972	1,017

Lampiran 7. Analisis Statistik Penambahan WCW/DCW Batch 3

Tests of Normality

	Kadar_Sorbitol	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
Penambahan_Berat_Sel_Kering_Pasca_96_Jam	S0	,334	3	.	,860	3	,266
	S30	,289	3	.	,927	3	,479
Penambahan_Berat_Sel_Basah_Pasca_96_Jam	S0	,208	3	.	,992	3	,828
	S30	,278	3	.	,940	3	,529

a. Lilliefors Significance Correction

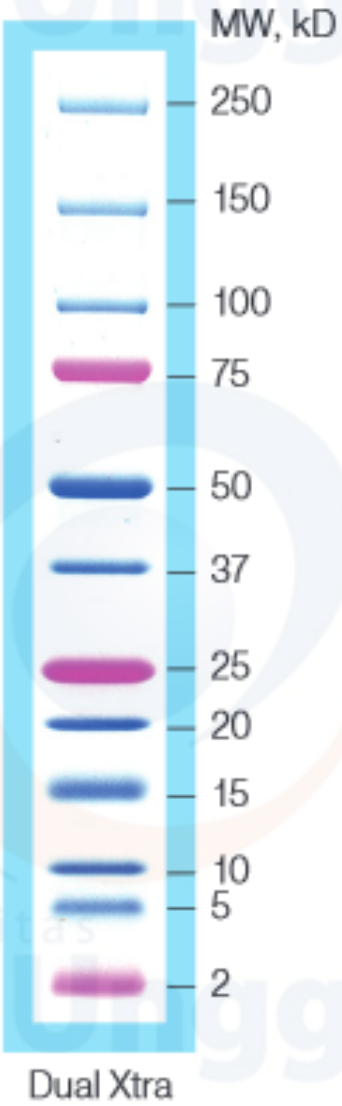
Hasil uji normalitas penambahan WCW/DCW batch 3

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
Penambahan_Berat_Sel_Basah_Pasca_96_Jam	Equal variances assumed	1,521	,285	-2,823	4	,024	,048	-,329000	,116548	-,652588	-,005412
	Equal variances not assumed			-2,823	3,087	,032	,064	-,329000	,116548	-,694090	,036090
Penambahan_Berat_Sel_Kering_Pasca_96_Jam	Equal variances assumed	4,300	,107	-11,105	4	<,001	<,001	-,230333	,020742	-,287922	-,172745
	Equal variances not assumed			-11,105	2,392	,002	,004	-,230333	,020742	-,306919	-,153747

Hasil uji homogenitas dan uji T-Test penambahan WCW/DCW batch 3

Lampiran 8. Ukuran Protein Marker



Ukuran marker (penanda) protein *dual xtra standard*
(Bio-Rad, 2010)
Keterangan:
MW (*Molecular Weight*); kDa (kilodaltons)

Lampiran 9. Analisis Statistik intensitas pita protein (Area *ImageJ*) Batch 3

Tests of Normality

	Kadar_Sorbitol	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
Area_ImageJ	Area ImageJ S0	,322	3	.	,880	3	,323
	Area ImageJ S30	,293	3	.	,922	3	,460

a. Lilliefors Significance Correction

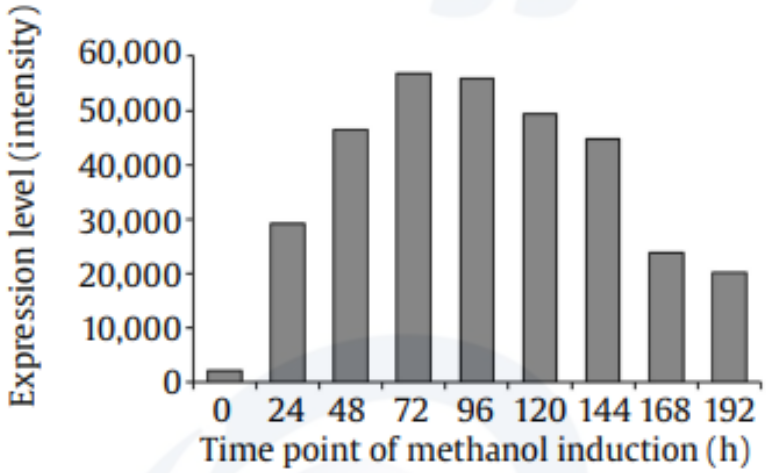
Hasil uji normalitas area *ImageJ* batch 3

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
Area_ImageJ	Equal variances assumed	,180	,693	-3,468	4	,013	,026	-5247492,333	1512951,329	-9448118,646	-1046866,021
	Equal variances not assumed			-3,468	3,856	,014	,027	-5247492,333	1512951,329	-9510613,057	-984371,609

Hasil uji homogenitas dan uji T-Test area *ImageJ* batch 3

Lampiran 10. Intensitas pita protein (Area *ImageJ*)



Intensitas pita protein (Area *ImageJ*) pada penelitian sebelumnya yaitu penelitian Nurdiani tahun 2022

(Nurdiani et al., 2022)